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RELATIVE TOXICITY TO SWINE OF LEAD ARSENATE SPRAY RESIDUE, LEAD ARSENATE, LEAD ACETATE, AND ARSENIC TRIOXIDE[1]

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INTRODUCTION

In regions where large amounts of lead arsenate sprays are applied to orchards, as a matter of routine, experience has led some handlers to believe that these sprays are only moderately toxic. Clinical observations both on man and animals lend some support to this view.[2] Although lead poisoning in horses is known to follow the long-continued consumption of hay grown between the rows of heavily sprayed trees (*3*),[3] the incidence of recognized lead or arsenic poisoning in persons handling the sprays and in animals fed unwashed apples or pears appears to be low, when the degree of exposure and the amount of spray residue ingested are considered. Controlled experiments (*7, 6, 4, 2*) also have indicated lead arsenate spray residue to be only moderately poisonous to sheep and swine. The acute lead arsenate poisoning of animals investigated by the writers (*5*) has followed the engorgement of very hungry animals on the leaves of heavily sprayed trees, and especially on the forage beneath the trees. This forage frequently is heavily encrusted with spray residue which accumulates from the slow dripping of the spray from the trees.

Facilities for washing apples and pears usually are fully employed in washing salable fruit. It therefore was desired to ascertain whether unwashed cull apples could safely be fed to pigs and whether the meat of animals fed such apples would contain significant amounts of lead and arsenic. Data on the storage of lead and arsenic in the body following long-continued ingestion of lead arsenic spray residues were also sought.

EXPERIMENTAL PROCEDURE

The experiment was planned for pig No. 1 to receive the maximum amount that it would consume of the spray residue from the peelings, including the stem and calyx ends, of heavily sprayed apples; pig 2 an equal amount of lead arsenate powder; pig 3 an equivalent

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[2] Personal communication from E. C. Reif. 1939.

[3] Italic numbers in parentheses refer to Literature Cited p. 165.

amount of lead as lead acetate; pig 4 an equivalent amount of arsenic as arsenic trioxide; and pig 5 one-fourth as much spray residue as pig 1. Pig 6 served as a control and received neither lead nor arsenic. All pigs received a mixed grain, tankage, bonemeal, and salt ration which was varied from time to time to meet the requirements of pigs of their weights.

A Chester White sow, previously used in lead arsenate toxicity experiments (*6*), in which she consumed 29 gm. of lead and 13.3 gm. of arsenic in the form of lead arsenate spray residue during a 20-week feeding period when she was fed the peelings of heavily sprayed apples, was bred to a boar of the same breed, and the pigs used in this experiment were farrowed January 19, 1941. The pigs were immunized against cholera and weaned at the age of 44 days. The feeding trials were started when the pigs were 92 days old, and continued for 103 days, from April 21, 1941, until August 2, when the supply of apples was exhausted. The pigs were trucked to a pasture August 2, and pig 3, which had received lead acetate, died the following day. Feeding was resumed on November 1 and continued for an additional 180 days, with the exception of December 27 to 30, inclusive, and March 2 to 5, inclusive, when pig 1 refused to eat apples. During these periods, apple feeding and medication of all the pigs was suspended. Both times, pig 1 was fed raw, lean meat to hasten the return of appetite. The pigs were killed April 30, 1942.

The number of apples, the weight of the apples and of the peelings fed pig 1, the calculated amounts of lead arsenate spray residue contained on these peelings, as well as the amounts of lead arsenate, lead acetate, and arsenic trioxide fed the different pigs during each 10-day period are shown in table 1.

In medicating pigs 2, 3, and 4, the substance to be fed was embedded in a quarter of an apple and the pig allowed to take this from the hand. Since the pigs were largely hand-fed, no difficulty was experienced in administering the lead arsenate, lead acetate, or arsenic trioxide by this technique.

FEEDING TRIALS

PIG 1, FEMALE

Pig 1 (fig. 1, *A*) received as much as it would consume of the peelings, including the stem and calyx ends of apples, which had been specially sprayed to obtain the maximum amount of lead arsenate spray residue. The peelings offered at each meal were weighed, and when not all were consumed the remainder was weighed and less was offered at the next meal. Aliquot samples of the peelings were placed in fruit jars and later analyzed for lead and arsenic. The calculated amounts served as the bases of lead arsenate, lead acetate, and arsenic trioxide fed pigs 2, 3, and 4, respectively.

During the first feeding period of 103 days, pig 1 consumed the peelings from 1,484 apples (table 1). The apples, before peeling, weighed 156 kg. (344.3 pounds) while the peelings and ends which were fed weighed 40.6 kg. (89.6 pounds) and contained 21.20 gm. of lead arsenate in the form of spray residue. During the second feeding period of 180 days, this pig consumed the peelings of an additional

6,123 apples, which before peeling weighed 851 kg. (1,876 pounds). The peelings fed weighed 175 kg. (386 pounds) and contained 93.64 gm. of lead arsenate. During the entire experiment, pig 1 was fed the peelings of 7,607 apples which weighed 1,007 kg. (2,220 pounds). The peelings consumed weighed 215.5 kg. (475 pounds) and contained 114.84 gm. of lead arsenate spray residue.

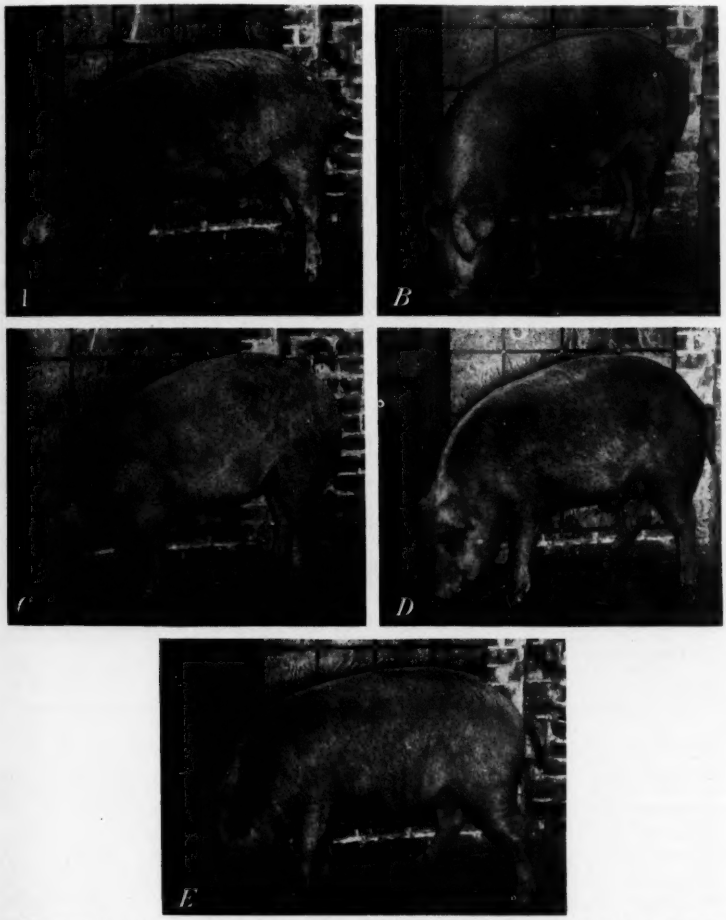

FIGURE 1.—*A*, Pig No. 1 after consuming the peelings, including stem and calyx ends, of 7,607 heavily sprayed apples containing 114.84 gm. of lead arsenate spray residue; *B*, pig 2, after consuming 103.416 gm. of lead arsenate powder; *C*, pig 4, after consuming 29.397 gm. of arsenic trioxide; *D*, pig 5, after consuming the peelings, including stem and calyx ends, of 1,831 heavily sprayed apples containing 28.14 gm. of lead arsenate spray residue; *E*, pig 6, the control, which received 215.5 kg. of peeled apples but no lead or arsenic. All photographs taken April 29, 1942.

TABLE 1.—*Amounts of peelings from sprayed apples, lead arsenate spray residue, lead arsenate, lead acetate, and arsenic trioxide consumed by experimental pigs*

10-day period commencing—	Pig 1				Pig 2	Pig 3	Pig 4	Pig 5		Pig 6
	Apples from which peelings were fed	Weight of apples [1]	Weight of peelings	Lead arsenate as spray residue on peelings	Lead arsenate	Lead acetate	Arsenic trioxide	Apples from which peelings were fed	Lead arsenate as spray residue	Peeled apples
1941	*Number*	*Kilograms*	*Kilograms*	*Grams*	*Grams*	*Grams*	*Grams*	*Number*	*Grams*	*Kilograms*
Apr. 21	134	13.45	4.83	2.13	2.125	2.326	0.521	53	0.53	4.83
May 1	206	21.38	5.41	2.84	2.842	3.110	.807	52	.71	5.41
11	130	14.64	4.76	1.77	1.771	1.944	.503	33	.44	4.76
21	107	13.17	3.06	1.52	1.420	1.572	.406	27	.38	3.06
31	130	15.24	3.67	1.70	1.365	1.502	.387	33	.42	3.67
June 10	130	13.37	3.30	1.75	1.381	1.521	.391	33	.43	3.30
20	130	15.52	3.39	2.11	2.110	2.340	.590	33	.53	3.38
30	127	13.74	3.42	2.11	2.110	2.340	.590	32	.53	3.43
July 10	130	13.76	3.57	2.11	2.110	2.340	.590	33	.53	3.57
20	130	14.78	3.47	2.11	2.110	2.340	.590	33	.53	3.47
30	130	7.12	1.75	1.05	1.055	1.170	.295	16	.26	1.75
Total, Apr. 21–Aug. 2	1,484	156.17	40.63	21.20	20.399	22.505	5.670	378	5.29	40.63
Nov. 1	127	18.27	4.05	1.98	1.696	.....	0.441	32	0.49	4.05
11	158	21.34	4.11	2.49	2.200	.....	.630	39	.63	4.11
21	160	22.36	4.92	2.51	2.200	.....	.630	40	.63	4.92
Dec. 1	179	22.91	4.76	2.72	2.326	.....	.646	45	.68	4.76
11	180	23.81	4.97	2.83	2.830	.....	.810	45	.71	4.97
21	379	51.08	13.20	5.98	1.884	.....	.540	95	1.50	13.19
31	400	51.16	12.94	6.28	5.354	.....	1.535	100	1.57	12.94
1942										
Jan. 10	386	52.60	11.24	6.07	6.280	.....	1.800	96	1.52	11.24
20	400	56.55	11.75	6.28	6.280	.....	1.800	100	1.57	11.75
30	400	55.10	11.24	6.28	6.280	.....	1.800	100	1.57	11.24
Feb. 9	400	53.94	11.52	6.28	6.280	.....	1.800	100	1.57	11.52
19	400	55.73	11.11	6.28	6.280	.....	1.800	100	1.57	11.11
Mar. 1	414	63.20	11.70	6.50	6.280	.....	1.800	103	1.62	11.70
11	468	66.22	13.47	7.33	6.280	.....	1.800	116	1.83	13.47
21	602	80.66	14.70	9.43	6.280	.....	1.800	75	1.18	14.70
31	310	43.07	8.10	2.43	4.553	.....	1.305	77	1.22	8.10
Apr. 10	400	56.26	10.53	6.32	3.454	.....	.990	100	1.58	10.53
20	360	56.60	10.57	5.65	6.280	.....	1.800	90	1.41	10.57
Total, Nov. 1–Apr. 29	6,123	850.86	174.88	93.64	83.017	.....	23.727	1,453	22.85	174.87
Total fed both periods	7,607	1007.03	215.51	114.84	103.416	22.505	29.397	1,831	28.14	215.50

[1] Pigs 2, 3, 4, and 6 received the same weight of peeled apples that pig 1 consumed of apple peelings.

PIG 2, MALE

Pig 2 (fig. 1, *B*) was fed peeled apples in the same amount as the peelings and stem ends consumed by pig 1. In addition, it was planned to feed this pig the same amount of lead arsenate in gelatin capsules as pig 1 received in the form of spray residue. Actually, the amount could be only approximated, since it was not possible to predict the exact number of apples pig 1 would eat. During the first feeding period, pig 2 consumed 20.4 gm. of lead arsenate; during the second feeding period, 83.0 gm., or a total of 103.4 gm. of lead arsenate powder during the experiment.

PIG 3, FEMALE

Pig 3 was fed exactly as was pig 2, except that this pig received the same amount of lead in the form of lead acetate that pig 2 received in

the form of lead arsenate. During the first feeding period, pig 3 was fed 22.5 gm. of lead acetate. This pig died in pasture August 3. No autopsy was performed.

PIG 4, FEMALE

Pig 4 (fig. 1, *C*) was fed exactly as were pigs 2 and 3, except that it received the same amount of arsenic in the form of arsenic trioxide that pig 2 received in the form of lead arsenate. During the first feeding period, pig 4 received 5.670 gm. of arsenic trioxide; during the second period, 23.727 gm., or a total of 29.397 gm. during the experiment.

PIG 5, MALE

Pig 5 (fig. 1, *D*) was fed one-fourth as much of the peelings of heavily sprayed apples as was eaten by pig 1. This pig was included in the experiment because it was expected that pig 1 might succumb to the excessive amount of lead arsenate spray residue fed. Pig 5 consumed the peelings and ends from 378 apples during the first period, and from 1,453 apples during the second period, or a total of 1,831 apples. The calculated intake of lead arsenate in the form of spray residue being 5.29 gm. during the first period and 22.85 gm. during the second period, the total of 28.14 gm. lead arsenate consumed was in the form of spray residue.

PIG 6, MALE

Pig 6 (fig. 1, *E*), which served as a control, received the same weight of peeled apples that pig 1 did of apple peelings and ends. It did not receive spray residue, arsenic, or lead.

CLINICAL RESULTS OF FEEDING LEAD ARSENATE SPRAY RESIDUE, LEAD ARSENATE, LEAD ACETATE, AND ARSENIC TRIOXIDE

Daily observations of the experimental animals, their weight gains (table 2) and blood studies, indicate that the amounts fed of lead arsenate spray residue, lead arsenate, or arsenic trioxide had no appreciable influence on the pigs receiving them. At the end of the first feeding period (August 2), pig 5 had made the greatest gain and pig 4 the least. At the conclusion of the experiment, the five surviving pigs all weighed between 270 and 280 pounds and all appeared to be healthy.

Pig 3, which received lead acetate, appeared in good clinical condition at the end of the first feeding period on August 2, when the pigs were trucked to a pasture, but this pig died the following day.

Blood studies made at various times during the feeding trials revealed no consistent or significant deviation from normal. The averages of 8 blood examinations made between March 29 and April 22, 1942, showed pig 1 to have an average red cell count of 6,530,000, a white cell count of 17,775, and a hemoglobin concentration of 14 percent. The average values for pig 2 were 6,650,000 red cells, 13,580 white cells, and hemoglobin, 12.6 percent; for pig 4, 6,956,000 red cells, 18,775 white cells, and hemoglobin 13.6; for pig 5, 7,466,000 red cells, 14,543 white cells, and hemoglobin 14.7; and for pig 6, 7,190,000 red cells, 16,180 white cells, and a hemoglobin concentration of 12.8.

TABLE 2.—*Weights at different dates and ages of experimental pigs consuming arsenic as spray residue or in other form*

Date	Age	Pig 1 (female)	Pig 2 (male)	Pig 3 (female)	Pig 4 (female)	Pig 5 (male)	Pig 6 (male)
		Spray residue	Lead arsenate	Lead acetate	Arsenic trioxide	Spray residue	Control
1941	*Days*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Mar. 4[1]	44						
14	54	28.5	29.5	23.5	29	28	26.5
Apr. 4	74	30.25	32.25	27.5	35	34.25	31
25	95	39	41	35.5	42	42	29
May 24	125	45	47	44.5	45.25	49.5	48.75
Aug. 2	195	66	70.50	70.5	61.5	78.25	68.25
2[2]							
3[3]	196						
Oct. 27[4]	281						
Nov. 1	286	162.5	157		150	161	167
1[5]							
1942							
Apr. 30	466	270	276		270	270	280

[1] Pigs immunized with hog cholera vaccine.
[2] Pigs sent to pasture.
[3] Pig 3 died.
[4] Pigs returned to experimental pens.
[5] Experimental feeding of pigs resumed.

ANALYSES AND AUTOPSY

The lead and arsenic residues were determined on selected samples of the apples used in these experiments. The apples were peeled, and the peelings digested with nitric and sulfuric acids. The lead was precipitated from the digest as the sulfate, the precipitate leached with a solution of ammonium acetate, and the lead determined gravimetrically as the chromate (*8*). Arsenic was determined in the filtrate from the lead sulfate precipitation by the bromate method (*1*).

The livers, hearts, spleens, and samples of blood and intestinal contents were dried in an oven, and lead was determined in the dried material after ashing at 500° C. The lead was determined colorimetrically by the diphenylthiocarbazone method (*1*). Arsenic was determined by the bromate method (*1*). Sections of bone cut from the radius and the ulna were extracted with alcohol and ether before weighing for analysis.

No significant pathological changes were noted at autopsy. The content of lead and of arsenic in the various organs is shown in table 3. The heart, blood, and bones of pig 1 contained more lead than did those of pig 2. However, pig 1 had received approximately 11 percent more lead arsenate. Pig 5, which had received approximately one-fourth as much lead arsenate spray residue as pig 1, had two-thirds as much lead in the blood and more than one-half as much in the spleen. In the bones, where the greatest amounts of lead were stored, the concentration is roughly in proportion to the intake.

The concentration of arsenic in the bodies of pigs 1, 2, and 5 is also roughly proportional to the arsenic intake. In contrast, pig 4, which received the same amount of arsenic in the form of arsenic trioxide as pig 2 in the form of lead arsenate powder, and approximately 88 percent as much arsenic as pig 1 in the form of lead arsenate spray

residue, showed significantly less arsenic in the liver, heart, spleen, and blood.

TABLE 3.—*Amount of lead and arsenic found in body parts of experimental pigs*

Pig No.	Micrograms of lead per gram of original sample						Micrograms of arsenic per gram of original sample				
	Intestinal contents [1]	Liver	Heart	Spleen	Blood	Bones [1]	Intestinal contents [2]	Liver	Heart	Spleen	Blood
1	109	18.5	1.3	1.9	0.6	240	45	2.4	1.8	1.3	1.2
2	118	25.3	.7	2.4	.5	192	46	3.0	.8	1.3	.7
4	7	.9	.1	.7	.3	8.0	42	1.2	.3	.3	.4
5	26	2.9	.4	1.0	.4	49.3	24	.3	.1	.3	.3
6	6	1.1	.3	.7	.2	8.9	4	.3	.2	.5	.1

[1] Lead per gram of dry sample.
[2] Arsenic per gram of dry sample.

SUMMARY

The data obtained from the feeding trials described in this paper indicate that lead arsenate spray residues are much less toxic to swine than has generally been supposed. One pig consumed the spray residue from 1,007 kg. (2,220 pounds) of heavily sprayed apples which contained 114.8 gm. of lead arsenate in the form of spray residue. This amount is greatly in excess of that which would be consumed by pigs receiving cull apples or pears fed according to commercial practices. Nevertheless this pig gained in weight approximately as well as the control pig. No abnormalities were apparent in the blood studies of the pigs.

Data presented (see table 3) show that of the edible portions of pigs 1 and 2, which were fed large quantities of lead arsenate, only the livers contained more lead than the 7.14 parts per million limit set by the Food and Drug Administration of the Federal Security Agency [4] for spray residue on apples and pears, while none of the organs analyzed contained more than the limit of 3.57 parts per million set for arsenic trioxide.

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THE NUTRITIVE VALUE OF COTTONSEED MEAL, SOYBEAN MEAL, AND PEANUT MEAL WHEN USED SEPARATELY AND TOGETHER TO SUPPLEMENT THE PROTEIN OF PRAIRIE HAY IN EXPERIMENTS WITH STEERS [1]

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INTRODUCTION

The use of high-protein feeds by livestock feeders to supplement the protein of many common roughages for beef cattle is a general practice based on sound nutrition principles. Protein supplements, as a rule, effect a marked increase in the feed intake of animals on fibrous rations. By providing a better nutrient balance in a ration they increase the efficiency of utilization of the major feed nutrients. Maximum use can be made of available roughage when fed in this manner.

For a long time, cottonseed meal has been a standard protein supplement for cattle on the range and in the feed lot. In recent years soybean meal of equal protein content has been used in various forms. More recently, peanut meal has become available in some localities in sufficient quantity to attract the attention of cattlemen. Question has naturally arisen as to the relative protein value of these supplements when used alone and as a mixture to supplement low-protein rations.

Knowledge of the nutritive value, for cattle, of the protein in these meals is limited largely to the results of feed-lot tests so conducted as to measure the over-all nutritive effects of the added supplement. Although of practical value, these trials are frequently not sufficiently well controlled to bring out possible differences in protein quality. By means of nitrogen balance studies, however, quantitative measurement can be made of the utilization of protein from different sources, and the efficiency of the utilization can be determined in both digestion and metabolism. The utilization by ruminants of the protein in soybean meal has been studied in this manner by Harris, Work, and Henke (*5*),[2] Swanson and Herman (*13*), and Miller and Morrison (*7*). The general methods of investigation with permissible modifications have been discussed by Mitchell (*10*) in a recent review.

The primary purpose of the investigation reported here was to determine whether or not the protein furnished by a combination of cottonseed meal, soybean meal, and peanut meal is of higher nutritive value for the maintenance and growth of cattle than an equivalent amount of protein furnished by any one of these three supplements.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 176.

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The plan was to feed the meals as supplements to a basal ration of prairie hay of low-protein content. The procedure provided for determination of the apparent digestibility of the nutrients in the individual feeds and in the complete rations. The amount of nitrogen absorbed and retained by the animals on the various rations was to be used to calculate the biological value of the proteins furnished by each supplement in combination with the basal ration. Although this procedure does not differentiate between the nitrogen in the supplement and that in the basal ration, it brings out differences between rations most likely ascribable to differences in the quality of their protein. Also, it has the possible advantage of providing the animals with natural feeds which undoubtedly influence the type of microorganismal growth prevailing in the rumen.

EXPERIMENTAL PROCEDURE

Nitrogen balance experiments were conducted on four yearling Shorthorn steers purchased on the open market. Feces and urine were collected during 10-day periods following preliminary feeding periods of 10 days' duration or longer. The feces as voided were caught in a gutter and immediately transferred by an attendant to closed metal containers. The feces were weighed daily and a 2-percent sample was preserved with thymol at 0° C. Urine was collected by means of a rubber funnel connected to a hose leading to a container below the false bottom of the stall. The urine was preserved during the period by the addition of sulfuric acid and thymol.

The steers were weighed at the beginning and again at the close of each collection period. They were provided with water twice daily and given approximately 25 gm. of salt with each feeding. The basal ration consisted of 4.54 kg. (10 pounds) of prairie hay daily. The supplements were added at a level of 454 gm. daily, which is the minimum amount commonly used in range feeding. The daily allowance of feed was divided into equal portions and fed at approximately the same time each night and morning. The hay was fed after the supplements had been consumed. Any hay refused during a collection period was ground and analyzed and was deducted from the hay offered the steers.

The prairie hay was locally grown and had been cut in August. Little bluestem grass (*Andropogon scoparius*) predominated in the hay but there were traces of big bluestem (*A. furcatus*), Indian grass (*Sorghastrum nutans*), sweetgrass (*Anthoxanthum odoratum*), and weeds. The cottonseed meal was obtained from a crusher in Mississippi and was sold to contain 41 percent of protein. The peanut meal and soybean meal, which had been prepared by the hydraulic process, were obtained from a local crusher. The soybeans from which the soybean meal was processed were obtained from a northern State.

The chemical composition of the feeds is given in table 1. The analyses of feeds and feces were made on air-dry samples by official methods of the Association of Official Agricultural Chemists. (*1*) Nitrogen was determined in triplicate on 10-gm. samples of fresh feces and on 5-ml. samples of urine (*3*).

TABLE 1.—*Percentage composition of feeds and rations*[1] *used in digestion and nitrogen-utilization studies with steers*

Description of feed or ration	Dry matter	Crude protein (N × 6.25)	Ether exract	Crude fiber	N-free extract	Organic dry matter
Feed:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Prairie hay	90.35	3.56	1.90	29.15	48.22	82.23
Cottonseed meal	92.37	41.37	5.60	9.57	28.13	84.67
Peanut meal	93.50	42.50	8.55	12.01	25.07	88.13
Soybean meal	92.13	43.62	6.05	4.82	32.38	86.87
Protein mixture [2]	92.67	42.51	6.73	8.79	28.52	86.55
Ration:						
Hay and cottonseed meal	100.00	7.73	2.47	30.23	51.24	91.67
Hay and peanut meal	100.00	7.83	2.76	30.44	50.90	91.93
Hay and soybean meal	100.00	7.96	2.51	29.76	51.69	91.92
Hay and protein mixture [2]	100.00	7.85	2.58	30.14	51.26	91.83

[1] Composition of the rations is given on the dry-matter basis.
[2] The protein mixture consisted of one-third each of cottonseed meal, peanut meal, and soybean meal.

The four steers were simultaneously fed each ration and the rations were fed in the order listed from the top of table 1. Steer No. 3 accidentally hanged himself in the stanchion near the close of the preliminary period when he was receiving prairie hay and the protein mixture; consequently, his data were not available for the last collection period. The three remaining steers were rerun on the protein mixture supplemental ration with very similar results to those secured in the original digestion study.

The data on digestibility are expressed in the results as apparent digestion coefficients, while the efficiency of nitrogen utilization is expressed as percentage storage of total nitrogen and biological value. No direct determinations of metabolic nitrogen in the feces or of endogenous nitrogen in the urine were made on these steers. Swanson and Herman (*13*) have made extensive nitrogen utilization studies with heifers of approximately the same weight as the steers used in these trials. Their values of 5.3 gm. for the fecal metabolic nitrogen per kilogram of dry matter intake and .712 $W^{0.42}$ kg. for the grams of urinary endogenous nitrogen obtained in the feces are similar to that used by Miller and Morrison (*7*) and Harris and Mitchell (*4*) for lambs. The data presented were treated statistically for significance by the analysis of variance method (*12*).

RESULTS

The apparent digestibility of the nutrients in the various rations fed is shown in table 2.

The average apparent digestibility of protein, ether extract, crude fiber, nitrogen-free extract, and organic matter in the unsupplemented prairie hay ration was 7.1 percent, 7.4 percent, 56.1 percent, 48.0 percent, and 48.2 percent, respectively. The digestibility of all nutrients was increased in the supplemented rations, the increases being statistically significant in all cases with one exception, that being crude fiber digestibility when peanut meal was the supplement fed. The increase in digestibility of protein and ether extract was especially noticeable. The average apparent digestibility of protein increased from 7.1 percent in the unsupplemented hay ration to 45 percent or more in the supplemented rations, while that of ether extract increased from 7.4 percent to over 27 percent. The nutritional significance of these values is discussed later.

TABLE 2.—*Apparent digestibility, digestible nutrients, and nutritive ratio of rations of prairie hay and of prairie hay supplemented with high-protein meals*

Supplement fed with prairie hay	Steer No.	Apparent digestibility of—					Digestible[1] nutrients	Nutrient[1] ratio 1:
		Crude protein (N × 6.25)	Ether extract	Crude fiber	N-free extract	Organic matter		
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
None	1	6.6	10.3	58.6	50.3	50.5	.....	.....
	2	12.5	11.6	58.7	51.5	51.2	.....	.....
	3	12.5	7.9	44.2	40.1	39.7	.....	.....
	4	−3.3	0	63.1	50.3	51.5	.....	.....
Average	.....	7.1	7.4	56.1	48.0	48.2	44.8	164.9
Cottonseed meal	1	47.0	27.2	65.4	59.4	59.4	.....	.....
	2	44.2	20.8	60.8	54.7	54.9	.....	.....
	3	45.2	27.0	57.2	53.5	53.3	.....	.....
	4	43.5	33.1	62.2	55.7	56.2	.....	.....
Average	.....	45.0	27.0	61.4	55.8	55.9	52.1	14.0
Peanut meal	1	50.6	34.9	61.4	57.3	57.4	.....	.....
	2	52.4	35.7	56.9	54.7	54.7	.....	.....
	3	50.1	32.3	54.1	51.8	51.8	.....	.....
	4	49.7	33.9	61.5	56.3	56.8	.....	.....
Average	.....	50.7	34.2	58.5	55.0	55.2	51.9	12.0
Soybean meal	1	52.7	34.2	65.2	57.6	59.0	.....	.....
	2	46.5	32.5	58.8	53.3	53.9	.....	.....
	3	47.7	27.4	54.4	50.4	50.9	.....	.....
	4	45.6	27.9	64.7	58.5	58.5	.....	.....
Average	.....	48.1	30.5	60.8	54.9	55.6	52.0	12.6
Protein mixture[2]	1	49.5	42.1	67.1	57.4	59.9	.....	.....
	2	47.8	36.0	60.3	53.7	54.9	.....	.....
	[3]3	.....	.....	.....	.....	.....	.....	.....
	4	48.9	34.7	63.6	55.6	57.1	.....	.....
Average	.....	48.7	37.6	63.7	55.6	57.2	53.7	13.1

[1] Calculations based on average feed consumed per steer.
[2] The protein mixture consisted of one-third each of cottonseed meal, peanut meal, and soybean meal.
[3] Date not available because of accident.

There were only small differences in digestibility of corresponding nutrients among the rations containing different protein supplements. The average apparent digestibility of protein, ether extract, crude fiber, nitrogen-free extract, and organic matter in the mixed supplement ration was 48.7 percent, 37.6 percent, 63.7 percent, 55.6 percent, and 57.2 percent, respectively. With few exceptions, these values were not significantly higher than those obtained with single supplements.

The apparent digestibility of protein was 45.0 percent in the cottonseed meal ration, 48.1 percent in the soybean ration, and 50.7 percent in the peanut meal rations. The difference of 5.7 percent between the two extremes was barely significant, a minimum difference of 5.7 being required for odds of 1:19.

The digestibility of ether extract was significantly higher in the protein mixture ration and peanut meal ration than in the cottonseed meal ration, the average digestibility of ether extract in the two latter rations being 34.2 percent and 27.0 percent, respectively.

The average digestibility of crude fiber in the protein mixture was significantly higher than the coefficients of 58.5 and 60.8 obtained

with the peanut meal ration and soybean meal ration, respectively, but was not significantly higher than the coefficient of 61.4 obtained with the cottonseed meal ration.

The digestibility of nitrogen-free extract in the supplemented rations varied less than one percentage unit from 55.0 percent. The digestibility of organic matter likewise varied only slightly from 56 percent.

The standard error of the difference in the foregoing comparisons of nutrient digestibility among rations was 2.66 for protein, 3.05 for ether extract, 3.57 for crude fiber, 2.40 for nitrogen-free extract, and 2.55 for organic matter.

The apparent digestibility of the protein in the various supplements, as differentiated from that of the protein of the entire ration, was calculated by difference from data secured in obtaining the values given in table 2. These calculated digestion coefficients are given in table 3.

TABLE 3.—*Coefficient of apparent digestibility of protein in supplements, as determined by differences in digestion trials with steers* [1]

Supplement	Digestibility of protein, as determined by difference with steer No.—				
	1	2	3	4	Average
Cottonseed meal	80.8	75.5	77.7	74.7	77.2
Peanut meal	86.6	89.9	84.4	85.5	86.5
Soybean meal	89.9	78.4	80.8	76.8	81.4
Protein mixture [2]	85.1	82.0	([3])	84.0	83.7

[1] Each supplement was fed in combination with prairie hay in the ratio of 1 part of supplement to 10 parts of hay.
[2] The protein mixture consisted of one-third each of cottonseed meal, peanut meal, and soybean meal.
[3] Data not available because of accident.

Inspection of table 3 shows that with the exception of the coefficients calculated for soybean meal protein there was good agreement between the coefficients calculated from data secured with different steers. More variation occurred in the coefficients calculated for soybean meal protein than in those calculated for protein in the other supplements. The average coefficients of apparent digestibility of protein in peanut meal, soybean meal, cottonseed meal, and a combination of the three meals were 86.5, 81.4, 77.2, and 83.7, respectively. The coefficient found by difference for peanut meal was significantly (highly) greater than that for cottonseed meal. The difference between the digestibility of cottonseed meal and soybean meal was not significant while the difference between cottonseed meal and the protein mix approached significance. The standard error of the difference was 2.97. The coefficients given by Morrison (*11*) for the digestibility of protein in these three meals, although larger, show the same trend; and when listed in the same order as given above they are 89, 85, and 81, respectively.

The data of the nitrogen balance studies are presented in table 4. The data show that there was considerable variation in nitrogen intake, due to variable feed consumption, when the steers were fed prairie hay alone. Nitrogen intake by different steers on this ration ranged from 19.8 gm. to 24 gm. daily. Fecal nitrogen excretion was

quite constant and in the case of steer 4 exceeded the nitrogen intake. Nitrogen balances were negative for all steers on the unsupplemented hay ration, the average total excretion of fecal and urinary nitrogen exceeding the average nitrogen intake by 13.2 gm. daily. The average biological value of the protein of prairie hay when fed alone was 65.

TABLE 4.—*Nitrogen balance data showing utilization by steers of nitrogen in prairie hay and in rations of prairie-hay and high-protein meal supplements* [1]

Steer No.	Body weight			Dry matter intake	Nitrogen intake	Fecal nitrogen	Absorbed nitrogen [2]	Total urinary nitrogen	Feed nitrogen utilized [3]	Total nitrogen stored	Nitrogen stored	Biological value
	Initial	Final	Average									
	Kg.	*Kg.*	*Kg.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Percent*	
PRAIRIE HAY												
1	268	273	270	3,718	22.9	21.4	21.2	17.6	11.1	−16.1	−70.3	52
2	281	280	280	3,844	24.0	21.0	23.4	15.2	15.8	−12.2	−50.8	68
3	269	273	271	3,488	21.7	19.0	21.2	11.7	17.0	−9.0	−41.5	80
4	306	303	304	3,255	19.8	20.4	16.7	14.7	9.9	−15.3	−77.3	59
Average												65
PRAIRIE HAY AND COTTONSEED MEAL												
1	270	274	272	4,488	55.6	29.5	49.9	18.9	38.5	7.2	12.9	77
2	277	276	276	4,479	55.6	31.0	48.3	22.9	32.9	1.7	3.1	68
3	265	268	267	4,504	55.7	30.5	49.1	20.4	38.1	4.8	8.6	74
4	303	299	301	4,510	55.8	31.6	48.1	21.4	34.5	2.8	5.0	72
Average												73
PRAIRIE HAY AND PEANUT MEAL												
1	263	269	266	4,484	56.4	27.9	52.3	25.1	34.1	3.4	6.0	66
2	270	277	273	4,478	56.3	26.8	53.2	24.5	36.2	5.0	8.8	68
3	262	266	264	4,517	56.7	28.3	52.3	22.8	36.9	5.6	9.9	71
4	290	304	297	4,522	56.6	28.5	52.2	24.5	35.5	3.7	6.5	68
Average												68
PRAIRIE HAY AND SOYBEAN MEAL												
1	283	284	284	4,516	57.5	27.2	54.2	21.3	40.5	9.0	15.7	75
2	280	281	280	4,487	57.7	30.7	50.3	22.7	35.4	4.0	7.0	70
3	265	275	270	4,516	57.5	30.1	51.3	21.6	37.2	5.8	10.1	73
4	298	297	298	4,498	57.3	31.2	49.9	24.9	32.8	1.2	2.1	66
Average												71
PRAIRIE HAY AND PROTEIN MIXTURE [4]												
1	279	285	282	4,519	56.7	28.6	52.1	21.3	38.4	6.8	12.0	74
2	279	281	280	4,519	56.7	29.6	51.1	21.7	37.0	5.4	9.5	72
3 [5]												
4	295	297	296	4,519	56.7	29.0	51.7	22.2	37.3	5.5	9.7	72
Average												73

[1] Dry matter intake and nitrogen values in grams represent average daily amounts.
[2] Based on an estimated value for the metabolic nitrogen in the feces.
[3] Based on an estimated value for the endogenous nitrogen in the urine.
[4] The protein mixture consisted of one-third each of cottonseed meal, peanut meal, and soybean meal.
[5] Data not available because of accident.

When prairie hay was supplemented with any one or a mixture of all three of the supplements used in this study, feed consumption was increased and a uniform nitrogen intake of between 55.6 and 57.7 gm. daily resulted. Nitrogen balances were positive and significantly higher in all trials with the supplemented rations. The average percentage of total nitrogen stored daily by steers on the cottonseed meal, peanut meal, soybean meal, and protein mixture rations was 7.4 percent, 7.8 percent, 8.7 percent, and 10.4 percent, respectively. Differences between these average values were not statistically significant.

The average biological value of the protein was 73 for the cottonseed meal ration, 68 for the peanut meal ration, 71 for the soybean meal ration, and 73 for the protein mixture ration. Differences between these values were not significant.

Considerable increase in hay consumption was noted when the steers were transferred from the all-hay ration to supplemented rations Because of the importance of hay as an economical source of feed nutrients the amount refused by the steers on each ration used in this study is shown in table 5. When the hay was fed alone at the rate of 4.54 kg. of air-dry hay daily, the amount of dry matter refused ranged from 254 gm. to 840 gm. daily. When the hay was supplemented with a protein concentrate refusals were small, the maximum amount refused being 44 gm. of dry matter daily.

TABLE 5.—*Average amount of prairie hay (dry matter) refused daily by steers on different rations*

Ration	Prairie hay offered (dry matter)	Prairie hay refused (dry matter) by steer No.—			
		1	2	3	4
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
Prairie hay	4,098	380	254	610	840
Prairie hay and cottonseed meal	4,098	30	38	13	7
Prairie hay and peanut meal	4,098	38	44	5	0
Prairie hay and soybean meal	4,098	0	29	0	18
Prairie hay and protein mixture [1]	4,098	0	0	[2]	0

[1] The protein mixture consisted of one-third each of cottonseed meal, peanut meal, and soybean meal.
[2] Data not available because of accident.

DISCUSSION

A small coefficient of apparent digestibility of protein is characteristic of all low-protein roughages. An average coefficient of only 7.1 percent was obtained in the present study with the unsupplemented prairie hay ration which contained 3.56 percent of crude protein. However, if a correction is made for the metabolic nitrogen in the feces based upon the value of 5.3 gm. of nitrogen per kilogram of dry matter consumed, a true digestibility coefficient of 96.4 percent is obtained. This value employed in nitrogen utilization studies relates to the amount of feed protein absorbed. The average true digestibility coefficients when calculated in this manner for the proteins in the supplemented rations were found to be 89.2 percent, 94.3 percent, 91.3 percent, and 92.6 percent, for the cottonseed meal, peanut meal, soybean meal, and protein mixture rations, respectively. It appears

evident, therefore, that the large difference in apparent digestibility of protein between the unsupplemented and the supplemented hay rations shown in table 2 was due not so much to differences in quality of protein as to differences in protein content of the rations and to factors that determine the excretion of metabolic nitrogen in the feces. These factors in relation to the protein content of the ration have been discussed by Mitchell (*8, 9*).

Acknowledgment of the foregoing facts need not involve the implication that coefficients of apparent digestibility are of little value in measurements of the protein value of feeds. On the contrary, they serve as practical measurements of the efficiency with which a feed can supply protein, without reference to its quality, to meet the overall requirements of the animal. The efficiency with which the animal utilizes that protein is another consideration. It has been pointed out in this connection (*8*), and recently demonstrated (*13*), that a negative correlation exists between the apparent digestibility of protein and its biological value.

The small coefficient obtained for the digestibility of ether extract in the unsupplemented hay ration has no important significance in itself, since the amount of ether-soluble material in hay is small and is made up largely of substances other than true fat, which have little, if any, value in supplying the fat or energy requirements of the animal (*2*). The materials which normally make up the ether extract of feces are for the most part unrelated to residual food fat or to the products of fat digestion, except perhaps in those instances when high fat rations are fed, or the fat is enclosed in cellular structures which resist fermentation and enzymic breakdown. The magnitude of the digestion coefficient for fat (ether extract) depends to a large degree on the fat content of the ration. It should be kept in mind, also, that a small error in the determination of fat as total ether-soluble material in the feces will produce a conspicuous error in digestion coefficients of less than 50 percent, and may easily change small positive coefficients to negative ones (*3*). In the present study, small but apparently significant differences were found in the digestibility of fat contributed by the different supplements.

The biological values reported here for the proteins in the supplemented rations are in fair agreement with those reported for the protein in soybean meal by Swanson and Herman (*13*) in experiments with heifers. They are higher than the values of approximately 60 obtained by Harris et al. (*5*) in experiments with steers, and by Miller and Morrison (*7*) in experiments with lambs. Johnson et al. (*6*) have pointed out that in rations containing 10 to 12 percent of protein, the biological value of the protein usually varies only slightly from 60. From this and other considerations these investigators advance the idea that the protein ultimately utilized by ruminants is largely micro-organismal protein regardless of the nature of the protein in the ration as consumed. There is nothing in the results of the present study that might be considered as contradictory to this conception of protein utilization. In fact, the similarity of the biological values obtained, support it; the magnitude of the values is related to the low protein content and wide nutritive ratio of the rations employed. Further, the data indicate that cottonseed meal, peanut

meal, and soybean meal when fed singly or in combination as supplements to prairie hay have about equal protein value as measured by protein storage and efficiency of utilization.

SUMMARY

Cottonseed meal, peanut meal, soybean meal, and a mixture of equal parts of the three meals were compared in nutritive value when fed to steers as supplements to a basal ration of prairie hay of low-protein content. The supplements were fed with the hay in the ratio of 1 part of supplement to 10 parts of hay. The dry matter of the hay contained 3.94 percent of protein and 44.8 percent of total digestible nutrients. The dry matter consumed in supplemented rations averaged 7.84 percent in protein and 52.7 percent in total digestible nutrients. The nutritive ratio of the supplemented rations averaged 1:12.9.

The digestibility of all nutrients was greater in the supplemented rations than in the unsupplemented prairie-hay ration. Differences among the supplemented rations in digestibility of corresponding nutrients were small. The apparent digestibility of protein was highest in the peanut meal ration, 50.7 percent, and lowest in the cottonseed meal ration, 45.0 percent. The difference between these two extreme values was barely significant. When calculated by difference the coefficients of apparent digestibility of protein in cottonseed meal, peanut meal, soybean meal, and the protein mixture were 77.2, 86.5, 81.4, and 83.7, respectively. The difference was significant in a comparison of peanut meal protein with cottonseed meal protein, while that between cottonseed meal and the protein mix approached significance.

The nitrogen intake of the steers on the supplemented rations ranged from 55.6 to 57.7 gm. daily. The efficiency of utilization of the nitrogen in digestion when expressed as true digestion coefficients was 89.2 percent for the cottonseed meal ration, 94.3 percent for the peanut meal ration, 91.3 percent for the soybean meal ration and 92.6 percent for the protein mixture ration.

The efficiency of utilization of the absorbed nitrogen when expressed as biological value was 73 for the cottonseed meal ration, 68 for the peanut meal ration, 71 for the soybean meal ration, and 73 for the protein mixture ration. Differences between these values were not significant.

The amount of nitrogen stored when expressed as a percentage of the total nitrogen intake showed no significant differences between rations.

It appears from a consideration of all data that despite the greater digestibility of protein in the peanut meal ration, utilization and storage of protein was about the same for each of the supplemented rations. There was no evidence to show that the protein furnished by a combination of the meals was superior in quality for yearling steers to that furnished by any one of them.

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COMPOSITION AND APPARENT DIGESTIBILITY OF THE CARBOHYDRATE AND OTHER CONSTITUENTS OF PEA AND LIMA-BEAN VINES WHEN FED TO SHEEP[1]

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INTRODUCTION

Pea vines and lima-bean vines[3] obtained as byproducts from pea and lima-bean canneries are annually available in large quantities and constitute a potential source of feed for farm animals. Pea vines have been used as a fodder for farm animals for many years. This material is relatively high in protein and cattle eat it readily. Lima-bean vines are available in considerably smaller quantities, and they have not been used to any great extent as stock feed.

The hemicelluloses constitute an important part of the undetermined fraction of hays and coarse fodders, conventionally designated as "nitrogen-free extract," and the extent to which they contribute to the maintenance of the animal is a matter of considerable importance. Pea and lima-bean vines are known to be especially rich in hemicelluloses, and these materials are therefore particularly suited for a study of the apparent digestibility of these carbohydrate complexes.

This paper reports a study of the composition and apparent digestibility of the carbohydrate and other constituents of pea and lima-bean vines preserved by artificial dehydration, with particular attention to the hemicelluloses and related constituents. Sheep were used as the experimental animals.

REVIEW OF LITERATURE

Rupel, Roche, and Bohstedt (*21*)[4] in 1931 reported that artificially dried pea-vine hay, when fed to cows, was equal in feeding value to good quality chopped alfalfa hay.

Tretsven (*17*) in 1936 reported two trials with pea-vine silage as a feed for dairy heifers. For milk production and growth, the pea-vine silage was worth approximately one-third the value of good quality alfalfa hay. When fed with suitable precaution, pea-vine silage had no influence on the flavor of the milk produced.

Johnson and Peterson (*7*) in 1940 reported the results of a study of the milk production and the vitamin A potency of the butterfat of a herd of dairy cows alternately receiving a ration that included

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[3] The terms "pea vines" and "lima-bean vines" as used in this paper include the empty pods as well as the vines.
[4] Italic numbers in parentheses refer to Literature Cited, p. 186.

25 pounds of pea-vine silage preserved with phosphoric acid and a dry ration in which alfalfa hay replaced the silage. The milk produced on the silage ration was markedly higher in carotene and vitamin A than that produced on the dry ration and the level of milk production was also more satisfactory.

Woodman and Evans (*22*) reported the results of digestion trials with sheep fed artificially dehydrated pea-pod meal and broad-bean-pod meal. On a dry-matter basis the pea-pod meal and bean-pod meal contained, respectively, 10.82 and 11.17 percent digestible crude protein; 0.94 and 0.62 percent digestible ether extract; 46.12 and 44.81 percent digestible nitrogen-free extract; and 10.63 and 10.37 percent digestible crude fiber. Woodman and Evans concluded that both products can be considered as desirable constituents in the rations of sheep.

Practically all previous investigators of the nutritive value of hemicelluloses have based their conclusions as to the apparent digestibility of these carbohydrates on the percentages of the total furfural afforded by the food consumed and the feces eliminated. Any method for the determination of hemicelluloses based on the assumption that only these carbohydrate complexes afford furfural when boiled with mineral acids is likely to lead to a rather serious error, and therefore much of the data on the digestibility of hemicelluloses must be accepted with considerable reserve. A critical résumé of the methods used for the quantitative estimation of hemicelluloses is presented elsewhere in this paper.

Fraps (*3*) found that the total pentosans of legumes were on an average digested better than the pentosans of nonlegumes. He also noted that the pentosans soluble in 0.02 N acid and alkali were better digested than the remaining pentosans.

Wille (*20*) in 1918 reported observations indicating the presence of a hemicellulose-splitting enzyme in the parotid glands and in the gall of cattle and swine, and in the stomach, pancreas, and small intestine of swine. Wheat bran, slices of the endosperm of lupine seeds, and cross sections of the rhizomes of *Molinia caerulea* were used as sources of hemicellulose materials.

Rippel (*15*) in 1921 reported the presence of enzymes in the seeds of *Lupinus angustifolius*, *Galium aparine*, and *Asparagus officinalis* capable of digesting the hemicelluloses of these seeds, and he took the view that the digestion effects observed in Wille's experiments were produced, in part at least, by the plant enzymes introduced with the hemicellulose material.

Shimizu (*16*) in 1921 reported results of digestion experiments conducted in vitro with extracts of macerated intestine and pancreas of dogs and rabbits. No monosaccharide could be found in the reaction mixture either by the phenylhydrazine or the Fehling reduction test and Shimizu concluded that there were no enzymes in such preparations capable of hydrolyzing hemicelluloses.

According to Manville, Bradway, and McMinis (*13*), galacturonic acid plays a role similar to that of glucuronic acid in the detoxication mechanism of the animal body. They, therefore, consider that foods containing pectin and hemicelluloses have a value separate and distinct from caloric considerations.

EXPERIMENTAL MATERIAL AND PROCEDURE

METHODS OF ANALYSIS

The pea and lima-bean vines used in this investigation were obtained from a cannery in Pennsylvania. As soon as the vines came off the viners, they were hauled by truck about 30 miles and dried in a commercial drier. The dry material was then shipped to the Beltsville Research Center at Beltsville, Md., where it was stored in a dry barn until used. Some of the lima-bean vines were moldy, but it is not known at what stage the mold developed.

Determination of Ammonium Oxalate Extractives and Furfural-Yielding Constituents

There is at present no satisfactory method for the quantitative estimation of hemicelluloses. The method most commonly used by agricultural chemists for the determination of hemicelluloses, generally called pentosans, is based on the fact that these substances when boiled with mineral acids yield furfural. This method, which was developed by Tollens and his school, is an empirical one. According to Kröber's (*9*) procedure, the furfural is precipitated with phloroglucinol and from the weight of phloroglucide obtained, the weight of pentosans is calculated.

While the method is quite satisfactory for the determination of pure pentose sugars or reasonably pure xylan or araban, there are objections to it when applied to the determination of hemicelluloses. In the first place, when a plant material is distilled with mineral acids, the furfural obtained is derived not only from the hemicelluloses, but also from pectic substances, so that the yield of furfural is frequently higher than would be the case if only hemicelluloses were present. Moreover, there is considerable difference in the percentage of furfural obtained from various hemicelluloses because of the variable composition of these carbohydrate complexes, so that it is impossible to correlate the percentage of furfural found with the percentage of hemicelluloses in the plant material under examination.

Accordingly, in the present investigation no attempt was made to determine the hemicelluloses as such. Instead, the sample under investigation was first freed from pectic substances by extraction with a hot 0.5-percent aqueous ammonium oxalate solution and then the percentage of furfural in the residual material was determined. The result thus obtained was calculated as the percentage furfural in the original unextracted sample and is recorded in tables 2, 3, and 4 under the heading, "Furfural-yielding constituents reported as furfural." It is realized, of course, that the percentage of furfural thus found is only an approximate indication of the percentage of hemicellulose in the sample, but since there is no satisfactory method for the direct determination of the carbohydrate complexes, the procedure followed here is believed to be the best available. The determination was carried out as follows:

To a 2-gm. sample contained in a 250-ml. centrifuge bottle, 200 ml. of a 0.5-percent aqueous ammonium oxalate solution (previously heated to 90° C.) was added, and the mixture was heated in a water bath maintained at 85° C. for 1 hour. From time to time the reaction mixture was stirred with a glass rod. At the end of the 1-hour period,

the reaction mixture was centrifuged and the clear supernatant solution was poured through a weighed sintered-glass crucible (porosity D). To the residue in the centrifuge bottle, 200 ml. of a 0.5-percent aqueous ammonium oxalate solution (previously heated to 90° C.) was added, and the mixture was heated again for 1 hour in the water bath maintained at 85° C. as previously described. The reaction mixture was centrifuged and the supernatant solution was filtered through the weighed sintered-glass crucible. This operation was repeated twice, making a total of four extractions. It was found experimentally that practically all the pectic substances were removed in the first two extractions. The residual material was then transferred to the weighed sintered-glass crucible, thoroughly washed with hot water, dried at 105° C., and weighed. The result was calculated on the basis of the original moisture-free material and is recorded in tables 2, 3, and 4 under the heading "Ammonium oxalate extractives."

The percentage of furfural in the residual material obtained in the above-described extraction with 0.5-percent aqueous ammonium oxalate solution was determined by the Tollens-Kröber procedure (*1*). The result obtained was calculated on the basis of the original moisture-free material and is reported in tables 2, 3, and 4 under "Furfural-yielding constituents reported as furfural."

Crude Cellulose

Crude cellulose was determined by the method of Kürschner and Hanak (*10*).

Determination of Pectic Substances

For the determination of pectic substances, the sample (5 gm.) was first exhaustively extracted with a hot 0.5-percent aqueous ammonium oxalate solution, the procedure previously described in connection with the determination of ammonium oxalate extractives being followed. To the filtered ammonium oxalate extract 3.5 volumes of 95-percent ethanol containing 10 ml. of concentrated hydrochloric acid per liter were added, and the mixture was allowed to stand overnight. It was then transferred portionwise to a 250-ml. centrifuge bottle, the mixture was centrifuged, and the clear supernatant solution was drawn off and discarded. The crude pectic material was shaken with 80-percent ethanol containing 10 ml. of concentrated hydrochloric acid per liter, centrifuged, and the clear solution was drained off and discarded. This operation was continued until the alcoholic washings no longer gave a test for oxalates or oxalic acid. To the pectic material in the centrifuge bottle, 40 to 50 ml. of water and 2 ml. of concentrated ammonium hydroxide solution were added, the mixture was stirred until the pectic substance was completely dissolved, and the solution was then transferred to a beaker. This solution was heated to boiling and filtered through a Hirsch type sintered-glass funnel of fine porosity. The centrifuge bottle was washed twice with 40 to 50 ml. of hot water and the washings were also passed through the sintered-glass funnel. A bell jar arrangement was used for filtering and for collecting the filtrate and washings. The combined filtrate and washings, after cooling to room temperature, were treated with 100 ml. of 0.4 percent of sodium hydroxide solution and were then allowed to

stand overnight. To this 50 ml. of N (approximately) acetic acid was added, together with an equal volume of a molar solution of calcium chloride, and the whole was boiled for 10 minutes. It was then filtered through a weighed soft filter paper (C. S. and S. No. 597) and the precipitate was washed with hot water until it was free of chlorides. It was dried at 105° C., weighed, and the result was calculated as percent of calcium pectate, in the moisture-free plant material.

All other analytical data recorded in this paper were obtained by the methods of the Association of Official Agricultural Chemists (*1*).

FEEDING EXPERIMENTS

For the feeding trials, conducted during the winter and spring of 1942, five Hampshire ewes were used; three were yearlings and two were 2-year-olds. Two ewes were used for each feed in trial 1. At the conclusion of this trial the feeds were reversed for each animal, and after a suitable preliminary period, a second trial was made. However, one pregnant animal (19U) that was used in the first trial (table 3) was replaced for the second trial (table 4). The animals were gradually accustomed to the rations to be used during the trials. For the first 8 days they were fed a hay mixture consisting of one part of pea or lima-bean vines and one part of alfalfa hay. They were also fed a grain mixture of one-half pound daily at the start, but this quantity was gradually decreased to none by the fourteenth day. The animals were then placed on a 13-day preliminary period, on the eighth day of which they were placed in the metabolism cages. The animal that replaced 19U was also gradually accustomed to the feed and had a preliminary period like the others.

During the preliminary period it was found that the sheep ate the pea or lima-bean vines more readily when they were dampened. The feed for each animal was weighed daily, and from the same feed source a daily portion was saved for a 10-day composite sample for analysis. The amount of feed given was so adjusted that the animals would have enough to eat and still there would be no excessive accumulation of rejected feed. The refused feed was collected once daily, and at the end of each 10-day trial it was weighed, dried if necessary, weighed again, ground, and sampled for chemical analysis. The pea vines were rather fine, dry, and dusty; the lima-bean vines were coarse and stemmy as well as dry and dusty. Moldy material was discarded.

Metal metabolism cages were used. The feces were retained on a fine copper screen and on alternate days they were collected from the screen. The feces were kept in a covered can, toluene being used as a preservative, and were stored in a refrigerator. At the end of each collection period, the total quantity of feces from each animal was spread out thinly on large sheets of paper, dried in a current of air at 40° C., ground, weighed, and a sample was removed for analysis. The feces dried within 48 hours without molding.

The urine was collected daily from the container kept under each metabolism cage and stored in large glass bottles. It was kept acid at all times by adding 1 ml. of a 10-percent acetic acid solution for each liter of urine collected. The final volume of urine was measured, mixed, and an aliquot was removed for analysis.

In all experiments the animals had unrestricted access to water.

EXPERIMENTAL RESULTS

The results obtained are recorded in tables 1 to 4.

TABLE 1.—*Gain or loss in weight and nitrogen retained by sheep fed on pea and lima-bean vines*

Fed pea vines			Fed lima-bean vines		
Sheep No.	Gain or loss in weight	Nitrogen retained	Sheep No.	Gain or loss in weight	Nitrogen retained
	Kilograms	*Grams*		*Kilograms*	*Grams*
6V [1]	+1.0	50.4	6V	+0.3	42.3
17U [2]	+.5	56.9	17U	−.8	30.2
18V	+1.6	76.4	18V	+.4	35.4
42V	−.2	69.1	19U	−1.7	20.1
Mean	+.7	63.2		−.4	32.0

[1] V = Yearling sheep.
[2] U = 2-year-old sheep.

TABLE 2.—*Percentage composition of the samples of pea and lima-bean vines that were fed and refused*

[Calculated on moisture-free basis]

Feed	Ash	Crude protein	True protein	Fat	Crude fiber	Nitrogen-free extract	Ammonium oxalate extractives	Furfural-yielding constituents reported as furfural	Crude cellulose (ash-free)	Pectic substances (as Ca pectate)
Pea vines:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Fed	12.81	16.44	11.11	2.03	23.63	45.09	39.59	6.45	23.85	4.28
Refused	9.57	10.70	6.82	1.28	37.55	40.90	32.39	8.90	32.92	3.33
Lima-bean vines:										
Fed	11.64	12.52	8.17	1.62	29.33	44.89	34.28	7.63	30.38	5.27
Refused	12.42	11.28	7.32	1.55	32.26	42.49	30.93	7.60	31.38	4.28

Table 1 shows the gain or loss in weight of and the nitrogen retention by the sheep used in the feeding trials. In the feeding trials with pea vines all the animals, except one, not only maintained their weight, but actually gained weight. The nitrogen retained ranged from 50.4 to 76.4 gm. The animals fed lima-bean vines, on the other hand, lost weight in two feeding trials, and barely maintained their weight in the other two. The nitrogen retained was considerably less in the feeding trials with lima-bean vines than in those with pea vines.

Table 2 shows the average chemical composition of the forage fed and of the feed refused. The percentage of crude protein in the pea vines was greater than in the lima-bean vines. In both materials, as was to be expected, the crude protein and pectic substances were lower, while the crude fiber and crude cellulose were higher, in the refused feed than in the feed offered. The nitrogen-free extract and the ammonium oxalate extractives in both materials were higher in the feed offered, however, than in the feed refused.

In tables 3 and 4 data are given on the dry matter and nutrients ingested, voided, and digested, and the mean apparent digestibility of the various constituents of pea and lima-bean vines, respectively. These tables show that the apparent digestibility of the dry matter

TABLE 3.—*Dry matter and nutrients of lima-bean vines as determined from the material offered and refused and in that ingested, voided, and digested by sheep*

[Weight in grams]

Feed for sheep No.—	Dry matter	Ash	Crude protein	True protein	Crude fat	Crude fiber	Nitrogen-free extract	Ammonium oxalate extractives	Furfural-yielding constituents reported as furfural	Crude cellulose (ash-free)	Pectic substances (as Ca pectate)
6V:											
Offered	16,000	1,729.6	1,937.6	1,260.8	244.8	4,547.2	6,692.8	5,171.2	1,166.4	4,704.0	710.4
Refused	4,591	531.6	472.0	299.3	63.4	1,466.8	1,821.2	1,323.1	328.3	1,404.8	164.4
Ingested	11,409	1,198.0	1,465.6	961.5	181.4	3,080.4	4,871.6	3,848.1	838.1	3,299.2	546.0
Voided	4,023	802.6	613.1	438.5	96.6	1,170.7	1,116.4	809.4	337.1	796.6	----------
Digested	7,386	395.4	852.5	523.0	84.8	1,909.7	3,755.2	3,038.7	501.0	2,502.6	----------
Digested (percent)	64.7	33.0	58.2	54.4	46.7	62.0	77.1	79.0	59.8	75.8	----------
17U:											
Offered	16,000	1,729.6	1,937.6	1,260.8	244.8	4,547.2	6,692.8	5,171.2	1,166.4	4,704.0	710.4
Refused	6,114	755.7	690.3	453.6	91.7	1,735.2	2,506.1	1,808.5	433.5	1,809.7	295.3
Ingested	9,886	973.9	1,247.3	807.2	153.1	2,812.0	4,186.7	3,362.7	732.9	2,894.3	415.1
Voided	3,545	741.3	536.0	396.0	76.9	1,034.8	984.4	764.3	282.2	698.4	----------
Digested	6,341	232.6	711.3	411.2	76.2	1,777.2	3,202.3	2,598.4	450.7	2,195.9	----------
Digested (percent)	64.1	23.9	57.0	50.9	49.8	63.2	76.5	77.3	61.5	75.9	----------
18V:											
Offered	16,000	1,796.8	1,856.0	1,214.4	246.4	4,336.0	6,897.6	5,211.2	1,144.0	4,496.0	886.4
Refused	3,410	386.0	351.6	230.9	50.1	1,087.1	1,359.9	956.5	251.0	1,029.8	131.6
Ingested	12,590	1,410.8	1,504.4	983.5	196.3	3,248.9	5,537.7	4,254.7	893.0	3,466.2	754.8
Voided	4,682	918.1	675.1	486.0	108.6	1,428.0	1,289.0	955.6	381.1	1,048.8	----------
Digested	7,908	492.7	829.3	497.5	87.7	1,820.9	4,248.7	3,299.1	511.9	2,417.4	----------
Digested (percent)	62.8	34.9	55.1	50.6	44.7	56.0	76.7	77.5	57.3	69.7	----------
19U:											
Offered	16,000	1,796.8	1,856.0	1,214.4	246.4	4,336.0	6,897.6	5,211.2	1,144.0	4,496.0	886.4
Refused	7,183	854.8	788.7	508.6	110.6	2,177.9	2,939.3	2,231.0	521.5	2,068.7	287.3
Ingested	8,817	942.0	1,067.3	705.8	135.8	2,158.1	3,958.3	2,980.2	622.5	2,427.3	599.1
Voided	2,841	570.5	449.4	340.1	65.9	811.1	812.2	645.8	225.6	534.1	----------
Digested	5,976	371.5	617.9	365.7	69.9	1,347.0	3,146.1	2,334.4	396.9	1,893.2	----------
Digested (percent)	67.8	39.4	57.9	51.8	51.5	62.4	79.5	78.3	63.8	78.0	----------
Average digested (percent)	64.8	32.8	57.0	51.9	48.2	60.9	77.4	78.0	60.6	74.8	----------

TABLE 4.—*Dry matter and nutrients of pea vines as determined from the material offered and refused and in that ingested, voided, and digested by sheep*

[Weight in grams]

Feed for sheep No.—	Dry matter	Ash	Crude protein	True protein	Crude fat	Crude fiber	Nitrogen-free extract	Ammonium oxalate extractives	Furfural-yielding constituents reported as furfural	Crude cellulose (ash-free)	Pectic substances (as Ca pectate)
6V:											
Offered	16,000	1,952.0	2,470.4	1,665.6	308.8	3,680.0	6,835.2	5,969.6	1,032.0	3,680.0	708.8
Refused	2,954	261.1	297.2	187.3	35.5	1,064.6	1,157.7	900.4	252.3	936.4	110.5
Ingested	13,046	1,690.9	2,173.2	1,478.3	273.3	2,615.4	5,677.5	5,069.2	779.7	2,743.6	598.3
Voided	4,772	1,285.6	772.6	549.7	126.0	1,085.2	1,246.9	1,037.0	281.5	820.8	.....
Digested	8,274	405.3	1,400.6	928.6	147.3	1,530.2	4,430.6	4,032.2	498.2	1,922.8	.....
Digested (percent)	63.4	24.0	64.4	62.8	53.9	58.5	78.0	79.5	63.9	70.1	.....
17U:											
Offered	16,000	1,952.0	2,470.4	1,665.6	308.8	3,680.0	6,835.2	5,969.6	1,032.0	3,680.0	708.8
Refused	4,782	482.0	554.2	347.6	62.2	1,628.7	1,887.5	1,512.1	406.0	1,434.6	156.8
Ingested	11,218	1,470.0	1,916.2	1,318.0	246.6	2,051.3	4,947.7	4,457.5	626.0	2,245.4	552.0
Voided	3,864	1,060.3	649.2	466.4	99.7	865.5	1,017.8	921.2	206.0	641.4	.....
Digested	7,354	409.7	1,267.0	851.6	146.9	1,185.8	3,929.9	3,536.3	420.0	1,604.0	.....
Digested (percent)	65.5	27.9	66.1	64.6	59.6	57.8	79.4	79.3	67.1	71.4	.....
18V:											
Offered	16,000	1,960.0	2,547.2	1,724.8	308.8	3,532.8	6,934.4	6,118.4	936.0	3,600.0	596.8
Refused	2,205	199.8	212.6	137.6	26.9	809.7	861.1	698.3	188.3	718.8	66.2
Ingested	13,795	1,760.2	2,334.6	1,587.2	281.9	2,723.1	6,073.3	5,420.1	747.7	2,881.2	530.6
Voided	5,318	1,369.9	869.5	595.6	143.6	1,291.2	1,363.0	1,198.7	322.3	989.1	.....
Digested	8,477	390.3	1,465.1	991.6	138.3	1,431.9	4,710.3	4,221.4	425.4	1,892.1	.....
Digested (percent)	61.4	22.2	62.8	62.5	49.1	52.6	77.6	77.9	56.9	65.7	.....
42V:											
Offered	16,000	1,960.0	2,547.2	1,724.8	308.8	3,532.8	6,934.4	6,118.4	936.0	3,600.0	596.8
Refused	3,955	343.7	385.2	249.2	47.5	1,464.1	1,539.3	1,199.2	337.4	1,257.7	108.4
Ingested	12,045	1,616.3	2,162.0	1,475.6	261.3	2,068.7	5,395.1	4,919.2	598.6	2,342.3	488.4
Voided	4,477	1,207.0	745.9	516.6	124.5	1,017.2	1,216.4	1,088.4	257.4	752.1	.....
Digested	7,568	409.3	1,416.1	959.0	136.8	1,051.5	4,178.7	3,830.8	341.2	1,590.2	.....
Digested (percent)	62.8	25.3	65.5	65.0	52.3	50.8	77.5	77.9	57.0	67.9	.....
Average digested (percent)	63.3	24.8	64.7	63.7	53.7	54.9	78.1	78.6	61.2	68.8	.....

of pea and lima-bean vines was about the same. The apparent digestibility (expressed as percent digested) of the crude protein, true protein, and crude fat of the pea vines was greater than that of the same constituents in lima-bean vines. The apparent digestibility of the nitrogen-free extract, ammonium oxalate extractives, and of the furfural-yielding constituents of both materials was about the same. The apparent digestibility of the crude fiber and crude cellulose in lima-bean vines, however, was greater than that of the same constituents in pea vines.

The apparent digestibility of the pectic substances in the two rations is not recorded in tables 3 and 4 because there was some uncertainty as to the extent of the digestibility of the pectic substances of the two plant materials submitted to feeding trials. When the several samples of feces were analyzed for pectic substances, by the method previously described, only small quantities of a dark precipitate were obtained in most cases. This material did not have any of the properties of calcium pectate and the percentage of calcium varied from somewhat under 1 to about 1.5 percent. The material obtained was clearly not calcium pectate, but a mixture of amorphous substances. It would appear that the pectic substances in passage through the digestive tracts of the animals were either completely digested and assimilated or that they were changed or degraded to such an extent that they could no longer be precipitated and determined by the method used. Kertesz (*8*), as a result of his experiments with dogs and human subjects, concluded that pectins when taken orally are not attacked until they reach the large intestine where they are completely hydrolyzed by bacterial enzymes.

DISCUSSION

As mentioned earlier in this paper, there is at present no satisfactory method for the quantitative estimation of hemicelluloses. In the method used in this investigation, a separation was made between the two principal furfural-yielding constituents, namely, the pectic substances and the hemicelluloses. While the figures in the columns headed "Furfural-yielding constituents reported as furfural" in tables 2, 3, and 4 cannot readily be converted into absolute quantities of hemicelluloses, they do nevertheless bear a close and direct relationship to the actual hemicellulose content of the materials under investigation. The results of the feeding trials with pea and lima-bean vines indicate very definitely that a considerable portion of the hemicellulosic material ingested was apparently digested, at least to the extent that there was a definite removal or destruction of the furfural-yielding components, in the hemicellulose complex. It is, of course, not known to what extent these components of the hemicellulose complex were actually utilized by the sheep as sources of energy or for other purposes. Moreover, it must be remembered that in addition to the furfural-yielding components, namely, pentosans and uronic acids, many of the hemicelluloses contain also hexosans and the analytical method used did not and could not measure the extent to which these were utilized by the sheep. In other words, the hemicelluloses in the pea and lima-bean vines may have been digested to a greater extent than is indicated by the data on the digestibility of the furfural-yielding constituents in tables 3 and 4.

SUMMARY

Feeding trials were conducted with sheep fed exclusively diets of either pea or lima-bean vines.

The animals fed an exclusive diet of pea vines not only generally maintained their weight, but most gained some weight.

The animals fed an exclusive diet of lima-bean vines lost weight in two feeding trials while in two others they barely maintained their weight.

The respective mean percentages of the apparent digestibility of the various constituents of pea and lima-bean vines were as follows: Crude protein, 64.7 and 57.0 percent; true protein, 63.7 and 51.9 percent; crude fat, 53.7 and 48.2 percent; crude fiber, 54.9 and 60.9 percent; nitrogen-free extract, 78.1 and 77.4 percent; ammonium oxalate extractives, 78.6 and 78.0 percent; furfural-yielding constituents, 61.2 and 60.6 percent; and crude cellulose, 68.8 and 74.8 percent.

The pectic substances of pea and lima-bean vines in passage through the digestive tracts of sheep were either completely digested or were changed or degraded to such an extent that they could no longer be determined in the feces by the calcium pectate method.

A considerable portion of the hemicellulosic complexes of the pea and lima-bean vines were apparently digested by the sheep, at least to the extent that they suffered a definite removal or destruction of the furfural-yielding components.

In these trials pea vines were found to be a satisfactory forage feed for sheep, but lima-bean vines were inferior to pea vines as an exclusive diet for sheep.

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THE INFLUENCE OF α-NAPHTHALENEACETIC ACID SPRAY ON THE MATURITY AND STORAGE PHYSIOLOGY OF APPLES, PEARS, AND SWEET CHERRIES[1]

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INTRODUCTION

Since 1940 spraying with growth-promoting chemicals for the control of the harvest drop of apples and pears has become a rather general commercial practice in the fruit-growing districts of the Pacific Northwest. This procedure has reduced losses from the dropping of apples and pears during harvest, and has enabled the grower to extend somewhat his harvesting operations during periods of labor shortage, especially in large orchards. Hormone[2] sprays have also been used in special cases where light crops, light soils, or weak trees may contribute to excessive fruit drop. Such sprays can also be used to advantage on certain varieties of apples and pears wherever the amount of harvest drop has always been great or where the fruit develops color rather slowly.

With proper timing of application of the spray and harvesting of the fruit thereafter, hormone sprays have reduced the harvest drop of apples without noticeably impairing quality through advanced maturity. Recently there has been a tendency to permit hormone-sprayed Bartlett pears to remain on the tree longer than recommended for best storage life, in order to obtain maximum tonnage. In such cases overripening, scald, and core break-down have often become severe after short periods of storage.

Considerable information has been accumulated on the efficiency of various growth-promoting chemicals in reducing the harvest drop of different varieties of apples and pears. Methods of applying these substitutes, their effective concentration, and the degree to which they control fruit drop have been studied. Little information has been reported, however, as to the effect of these chemicals on the keeping quality and storage physiology of the fruit itself. Persistent

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[2] Hormones are technically described as substances produced in one part of an organism and transferred to another part where they influence certain specific physiological processes. In conformity to popular usage, the word "hormone" has been employed in this paper to include certain growth-promoting chemicals that influence plant response.

reports of increased ripening of Bartlett pears following the use of a hormone spray have been made by both growers and canners. Whether this observed response is caused by advanced maturity due solely to delayed picking or to a specific stimulation of the fruit itself by the hormone spray is not known.

The present work was undertaken to study the harvest maturity and storage physiology of Winesap and Delicious apples (*Malus pumila* Mill.), Bartlett pears (*Pyrus communis* L.), and Bing cherries (*Prunus avium* L.) as influenced by the harvest application of α-naphthaleneacetic acid. The study included data on the amount of fruit drop at different stages of maturity from sprayed and unsprayed portions of the same trees. Soluble pectins, organic volatile constituents, respiratory intensity, internal break-down, and moisture losses were measured in studying the storage physiology of the experimental fruit.

REVIEW OF LITERATURE

Interest in plant growth-promoting chemicals has been greatly stimulated by the report of Gardner, Marth, and Batjer (*9*)[3] that these substances reduce the harvest drop of apples. No attempt will be made to review the numerous papers pertaining to this subject. Published reports relative to the effect of these hormone sprays on the maturity and storage qualities of the fruit itself are decidedly limited.

Burkholder and McCown (*4, p. 120*) stated: "The effect of the spray is merely to delay the natural chemical changes in the cell wall substances resulting in separation of cells and ultimate drop of fruit." Gardner, Marth, and Batjer (*8*) found that the spray had no measurable effect on the photosynthesis or respiration of apple foliage and suggested that the effect of the spray was entirely localized in the area where abscission occurred. Ellenwood and Howlett (*6*) concluded that harvest sprays did not arrest the normal processes of maturity within the fruit itself. Childs (*5*) cautioned that harvesting of Delicious apples sprayed with a hormone should not be delayed longer than 1 week to 10 days after normal picking. He reported inconclusive results from the use of hormone sprays on Anjou (Beurre d'Anjou) and Bosc (Beurre Bosc) pears. His observations on Bartlett pears after the normal picking period indicated that the sprayed fruit ripened faster and dropped in greater numbers than the unsprayed fruit. Overholser, Overley, and Allmendinger (*14*) concluded that α-naphthaleneacetic acid spray did not influence the maturity of apples and pears when harvested at the proper stage of maturity.

In storage tests with six varieties of apples picked 1 to 2 weeks after an application of α-naphthaleneacetic acid, Haller (*13*) found no direct effect of the spray on firmness, decay, break-down, or scald. Batjer and Marth (*3*) cautioned that in summer varieties of apples water core and mealiness may occur in hormone-sprayed fruits allowed to remain on the tree even a few days beyond the normal harvest season. These authors suggested that with many varieties

[3] Italic numbers in parentheses refer to Literature Cited, p. 206.

of apples hormone sprays may be of greatest value to the grower in merely protecting him from losses prior to and during the normal harvesting period.

MATERIAL AND METHODS

SOURCE OF MATERIAL

Studies of the influence of α-naphthaleneacetic acid on the maturity and storage physiology of fruit were initiated at Wenatchee, Wash., in 1941. On September 24 of that year main leaders of a normal, vigorous 20-year-old Winesap tree received a thorough application of the chemical (0.001 percent in a 1 to 5,000 water solution of Vatsol as a spreader). The remaining leaders of the tree received no spray.

In collecting samples for the physiological and storage investigations care was taken to obtain fruits of uniform size, firmness, color, and exposure to sunlight. Nine apple boxes of loose fruit were harvested from both the sprayed and unsprayed portions of the tree on October 9, 173 days from full bloom. Each lot was well composited, packed in oil wraps in standard wooden apple boxes, and stored immediately at 31° and 36° F. Samples were removed for biochemical analyses at irregular intervals. Respiration studies were also made on duplicate 8-kg. samples of the sprayed and the unsprayed fruits at both temperatures throughout a storage period of approximately 7 months.

During the 1942 season these studies included observations on Delicious apples and Bartlett pears. On September 18, one-half of a large, heavily loaded 20-year-old Delicious tree was sprayed with α-naphthaleneacetic acid in the same concentration as used the previous season. Six boxes of loose fruit for experimental storage observations and for biochemical analyses were harvested from each half of the tree at two maturities. These were stored immediately at 31° F. The first picking was made on October 3, 164 days after full bloom and 15 days after application of the hormone spray, at what was considered, because of the lateness of the season, medium commercial maturity for the variety. No commercial pickings had been made in the orchard at that time. The second picking was made at an advanced stage of maturity on October 19, 31 days after application of the spray. A "clean-up" picking followed on November 6, when the fruit was decidedly overripe and of little commercial value.

The influence of hormone sprays on Bartlett pears was studied on fruits grown in two different orchards. The three trees chosen in orchard A were approximately 12 years old. They were vigorous, with well-defined main leaders, each of which bore a full load of fruit. The crop in orchard A was somewhat later in maturing than that on the large 20-year-old tree in orchard B, located in an early-producing district. The tree used in these studies bore a normal crop on two well-defined main leaders, one of which was sprayed with α-naphthaleneacetic acid (0.001 percent in a 1 to 5,000 water solution of Vatsol as a spreader) on August 11. A similar application of the growth chemical was applied to halves of the three trees in orchard A on August 17.

Dropped fruits from both the sprayed and the unsprayed leaders of

all trees were counted and segregated every few days into two classes according to maturity, as follows:

Ripe.—Pears that were yellow and soft. The finger and thumb could easily be pressed into the flesh.

Green.—Pears that were green and hard. They were not noticeably yielding to finger and thumb pressure.

Pickings for storage observations and for physiological studies were made at two maturities in orchard A and at one maturity in orchard B. A total of six apple boxes of loose fruit was harvested at each maturity from both the sprayed and the unsprayed lots. The first picking in orchard A was made on August 25, 8 days after application of the hormone spray and 132 days from full bloom, at a firmness of 16 pounds' pressure and a maturity that was considered satisfactory for canning. The second picking was made on September 15, 29 days after application of the hormone spray and at a stage of maturity too advanced for commercial handling. Fruit from orchard B was picked on September 3, 145 days from full bloom and 23 days after spraying with α-naphthaleneacetic acid. Maturity at this time was too advanced for best cannery requirements.

The hormone-spray work with Bartlett pears was continued during the 1943 season, when one well-spaced main leader on each of three trees received an application of 0.001 percent α-naphthaleneacetic acid with no spreader on August 24. Maximum air temperatures on the date of application and on the day following were 78° and 88° F., respectively.

None of the fruits had dropped from the experimental trees prior to spraying. Maturity at this time was considered optimum for cannery requirements; the fruit had an average firmness of 16.3 pounds' pressure. Records were kept of the daily drop of ripe and green fruits from all trees.

From the sprayed leaders of two trees, fruits comparable in number and maturity with normal drops from the unsprayed control leaders of the same trees were picked daily in order to determine what effect partial crop removal might have on the ripening of the remainder of the fruit on the tree.

Commercial pickings on comparable trees adjacent to those under test were made at a pressure test of 14.6 pounds on September 3. Harvest of the experimental fruit was purposely delayed until September 17, 151 days from full bloom, at which time it was well beyond acceptable maturity for satisfactory storage (firmness of 12.8 pounds' pressure). All lots of Bartlett pears for ripening, storage, and physiological studies were packed in standard pear boxes and stored at 31° F. on the day of harvest.

On June 14, 1943, α-naphthaleneacetic acid (0.001 percent concentration) was applied to one-half of a normal, average-bearing, 10-year-old Bing cherry tree. The fruit from both the unsprayed and the sprayed halves of the tree was harvested 1 week later. Samples from each lot of fruit were stored for 10 days at 31° and 45° F. Determinations of the soluble solids in the fruits and the moisture content of the stems were made at the time of harvest. Loss of weight in the fruits and the moisture content of the stems were also recorded after a storage period of 10 days.

METHODS

Respiratory measurements on 8 to 9 kg. of fruit were made by absorbing the liberated carbon dioxide gas in 0.3 N barium hydroxide according to the procedure described by Gerhardt and Ezell (*12*).

Soluble pectins were determined as calcium pectate (*7*), and the volatile content of the fruit was measured by aeration and oxidation of the steam distillate of the fruit tissue (*10*).

Firmness of the fruit tissue was recorded by means of the United States Department of Agriculture pressure tester with a 7/16- and a 5/16-inch plunger for apples and pears, respectively.

A small pocket-model Zeiss refractometer was employed for the measurement of soluble solids in the Bing cherries. Determinations of moisture in the stems were made by drying the samples to constant weight in a vacuum oven at 70° C.

EXPERIMENTAL DATA

WINESAP APPLES

During 1941 records were kept on the amount of harvest drop of fruit from the hormone-sprayed and the unsprayed leaders of the Winesap tree. Over a period of 15 days from date of application of the α-naphthaleneacetic acid until harvest, the fruit drop on the respective leaders of the tree was as follows: Sprayed fruits, 5.3 percent of the total on the leaders; unsprayed fruits, 20.8 percent. These figures show that the unsprayed leaders dropped approximately 4 times as much fruit as the sprayed portions of the tree.

Certain physiological responses of these two lots of fruit at various intervals during storage at temperatures of 31° and 36° F. are shown in table 1. It will be seen that there was no marked difference in the firmness, soluble pectins, or respiration of the sprayed and the unsprayed lots at harvest or during storage. Since the rate of respiration is a measure of the intensity of the physiological processes within the fruit and the soluble-pectin content reflects the degree of ripening, the data in table 1 show that the storage behavior of Winesap apples was not influenced by the harvest application of the hormone spray. In this experiment, when Winesap apples were harvested at a good stage of maturity within 15 days after application of α-naphthaleneacetic acid, loss from harvest fruit drop was reduced and the maturity of the remaining fruit on the tree was not affected.

The storage behavior of both the sprayed and the unsprayed lots was directly correlated with the storage temperature. At 36° F. the average decrease in firmness of all lots of fruit was approximately double that at 31°, while the average soluble pectin and respiratory activity were 61.8 and 58.2 percent greater, respectively, at 36° than at 31°.

DELICIOUS APPLES

During the season of 1942 α-naphthaleneacetic acid was applied to certain main leaders on a Delicious apple tree. Records were kept of the amount of harvest drop from the time of application of the hormone spray until completion of harvest. These data, based on the

total number of original fruits on the sprayed and unsprayed leaders, were as follows:

Treatment and date of harvest:	*Fruit drop (percent)*
Sprayed:	
Oct. 3	0.1
Oct. 19	6.0
Nov. 6	44.1
Unsprayed:	
Oct. 3	2.6
Oct. 19	25.9
Nov. 6	86.9

TABLE 1.—*Physiological changes in Winesap apples during storage at 31° and 36° F., as influenced by the harvest application of α-naphthaleneacetic acid, 1941*

Storage temperature (°F.) and period (days)	Sprayed fruit				Unsprayed fruit			
	Firmness (pressure test)	Soluble pectin	Organic volatiles ($Ce(SO_4)_2$ reduced per 100 gm. of fresh tissue)	Respiration (CO_2 per kilogram-hour)	Firmness (pressure test)	Soluble pectin	Organic volatiles ($Ce(SO_4)_2$ reduced per 100 gm. of fresh tissue)	Respiration (CO_2 per kilogram-hour)
31° storage:	*Pounds*	*Percent*	*Milligrams*	*Milligrams*	*Pounds*	*Percent*	*Milligrams*	*Milligrams*
0	17.8	0.0317	101.3		17.9	0.0302	78.6	
8				2.88				2.63
27				2.67				2.76
46	18.2	.0337	112.1	2.89	17.8	.0337	87.0	2.72
74	17.7	.0412	116.2	2.82	17.4	.0450	83.5	2.66
111	17.3	.0575	127.8	3.22	16.6	.0775	136.6	3.20
138	15.1	.0610	142.8	3.17	15.5	.0830	131.0	2.88
172	15.4	.1025	132.7	2.43	16.0	.0950	138.6	2.26
210	14.8	.1287	130.0	2.38	15.3	.0973	121.7	2.21
Average	16.6	.0652	123.3	2.80	16.6	.0659	111.0	2.66
36° storage:								
0	17.8	.0317	101.3		17.9	.0302	78.6	
8				4.42				4.52
27				4.37				4.25
46	18.1	.0510	140.2	4.18	17.5	.0450	106.3	4.30
74	16.1	.0750	146.2	4.40	16.1	.0615	110.4	4.16
111	14.2	.1192	142.8	4.55	14.5	.1187	137.8	4.45
138	14.4	.1325	169.3	4.44	14.3	.1412	141.7	4.28
172	14.6	.1575	169.7	3.65	14.1	.1720	138.6	3.93
210	12.6	.1700	154.9	4.76	12.5	.1795	148.8	4.57
Average	15.4	.1053	146.3	4.34	15.3	.1068	123.1	4.30

The first picking was made 164 days from full bloom; for 1942 this would be considered a midseason commercial harvest for Delicious. At that time no fruit drop of importance had occurred even from the unsprayed leaders and only an occasional trace of slight water core was found in the fruit. At the time of the second harvest, approximately four times as much fruit had dropped from the unsprayed as from the sprayed leaders. Most of the fruit drop from both the sprayed and the unsprayed leaders occurred after the second, or final, experimental picking. The effectiveness of α-naphthaleneacetic acid in preventing fruit drop extended over a period of 6 weeks. The fruit harvested on October 19 was flecked with vascular water core and was almost tree-ripe. In a random selection of 100 fruits cut at harvest, it was observed that the water-cored areas were more numerous, larger, and more confluent in the hormone-sprayed than in the unsprayed lots.

At the time of the "clean-up" picking in November, all fruits on the tree were overripe, mealy, and of little commercial value. None of this fruit was held for observation during storage.

Data on the influence of the harvest application of the hormone spray as related to the storage physiology of Delicious apples are given in table 2. It will be seen that firmness, soluble pectin, and respiratory rate of the sprayed and the unsprayed fruits from the first picking were similar throughout a storage period of 194 days at 31° F. In other words, the degree of ripeness of the fruit, as measured by its storage physiology, was not altered when harvest occurred within 15 days after application of the growth chemical. In this respect the data in table 2 confirm the results obtained with Winesap apples of the previous season.

When the fruit was permitted to hang on the tree for 31 days after application of the spray, data in table 2 show that the hormone-treated lot was more mature at harvest and riper during storage than comparable lots from the unsprayed half of the tree. The latter, especially during the first 90 days of storage, were more firm; they respired at a slower rate and had a smaller amount of soluble pectin in their tissues than the sprayed fruit. The average respiratory rate of the sprayed fruit was approximately 14 percent greater than that of the unsprayed sample.

TABLE 2.—*Physiological changes in Delicious apples during storage at 31° F., as influenced by the harvest application of α-naphthaleneacetic acid, 1942*

Picking and storage period (days)	Sprayed fruit				Unsprayed fruit			
	Firmness (pressure test)	Soluble pectin	Organic volatiles ($Ce(SO_4)_2$ reduced per 100 gm. of fresh tissue)	Respiration (CO_2 per kilogram-hour)	Firmness (pressure test)	Soluble pectin	Organic volatiles ($Ce(SO_4)_2$ reduced per 100 gm. of fresh tissue)	Respiration (CO_2 per kilogram-hour)
First picking (Oct. 3):	*Pounds*	*Percent*	*Milligrams*	*Milligrams*	*Pounds*	*Percent*	*Milligrams*	*Milligrams*
5	15.1	0.0350	47.2	2.28	15.1	0.0375	48.3	2.07
60	14.3	.0537	98.0	2.05	15.0	.0450	114.7	1.95
107	14.0	.0750	127.8	2.09	14.0	.0775	116.2	1.90
153	12.6	.0750	117.9	2.80	12.3	.0850	101.4	2.75
194	11.7	.0800	141.0	3.00	11.4	.0850	146.1	2.95
Average	13.5	.0637	106.4	2.44	13.5	.0660	105.3	2.32
Second picking (Oct. 19):								
8	13.7	.0600	109.6	2.50	14.9	.0500	64.7	2.26
44	12.0	.0620	100.6	2.55	15.0	.0510	94.0	2.24
91	11.6	.0675	113.1	3.14	13.3	.0600	105.1	2.69
137	11.6	.0525	112.9	3.65	12.5	.0600	112.0	3.22
178	11.7	.0612	156.1	4.02	11.8	.0562	140.1	3.48
Average	12.1	.0606	118.5	3.17	13.5	.0554	103.2	2.78

Observations were also made on the condition and dessert quality of the test lots of Delicious apples of both pickings during storage at 31° F. and after ripening at 65° for 5 days. When the fruit was examined in storage on March 1, the dessert quality and firmness of the sprayed and the unsprayed lots of both maturities were very

similar. Most of the water-cored areas in the fruits from the second picking had disappeared during storage. No stale flavor could be detected in any of the lots. The similarity in dessert quality and condition between treated and untreated lots of the first picking was maintained during the period of post-storage ripening. Both lots became somewhat stale in flavor, but neither showed any internal break-down.

After the fruit had ripened for 5 days, however, there were marked differences in condition and amount of break-down between the sprayed and the unsprayed fruit from the second picking. Seventy percent of the hormone-sprayed fruit showed severe internal break-down after post-storage ripening as compared with only 20 percent in the unsprayed lots. The presence of such large amounts of break-down in this fruit after ripening is all the more striking when comparable lots in storage at 31° F. showed no evidence of this disorder. This fact suggests that, even with the disappearance of water core during storage at 31°, the affected areas remain weakened and potentially susceptible to discoloration, excessive oxidative changes, and final break-down during prolonged cold storage or during subsequent ripening at higher temperatures.

The influence of the post-storage ripening temperature on the subsequent development of internal break-down in water-cored fruit is shown in figure 1. On March 1, 1943, hormone-sprayed Delicious apples of the second picking were cut in half while still in storage at

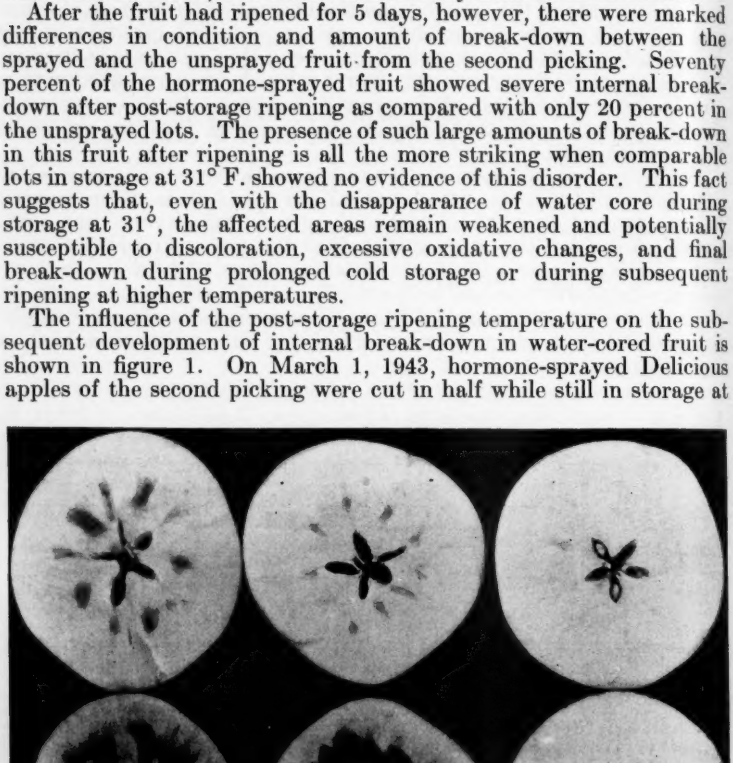

FIGURE 1.—Effect of ripening on the development of water core break-down in Delicious apples stored continuously at 31° F. for 5 months. On March 1, 1943, each apple was cut at its greatest transverse diameter. Upper and lower parts in each case are paired halves of the same apple after 5 days at 31° and 65°, respectively. It will be noted that the fruit at extreme right (check lot) was not water-cored.

31° F. The cut surface of each half of the fruit was immediately coated with a mineral oil-paraffin emulsion. Half of each apple was held at 31°, and the remaining half was removed to 65° and kept there for 5 days. At the end of that time the halves were reassembled for photographing. It is evident from figure 1 that internal break-down following water core developed rapidly at 65°, whereas at 31° it may have been potentially present but certainly was not visible. The figure also emphasizes the hazards of storing severely water-cored Delicious apples.

Since water core and break-down are closely associated with advanced maturity, the hormone-sprayed Delicious fruit of the second picking, with 70 percent break-down, must be considered more mature than the unsprayed lot. These observations on the ripening behavior of the various storage samples of Delicious apples supplement and confirm the results of the physiological studies reported in table 2. Both suggest that, when the fruit is sprayed with α-naphthaleneacetic acid and picking is delayed for a period of 30 days thereafter, ripening processes are stimulated. On the other hand, when the spray is applied not too far in advance (10 to 15 days) of the normal harvesting date, the grower can safely use it to retard the fruit drop from his Delicious trees without fear of noticeably stimulating the ripening rate.

BARTLETT PEARS

Maturity changes at harvest and ripening during storage are more easily distinguishable in Bartlett pears than in either Winesap or Delicious apples. This may result in part from the fact that pears are picked while still hard and green. They are generally more active physiologically than apples, and in ripening they quickly assume a yellow color and a soft buttery texture. Consequently, they are admirably suited to studies where maturity and ripening may be influenced by harvest application of hormone sprays.

During the 1942 season records were kept of the amount and maturity of the dropped fruit from the hormone-sprayed and the unsprayed Bartlett pears in two orchards of different ages and at different locations. These data are shown in table 3. Field records (not included in table 3) showed that, at the time of the first picking (August 25), an average of only 1.4 and 1.8 percent of the total fruit had dropped from the sprayed and the unsprayed leaders, respectively. During this period the drop was green fruit in both cases. All but approximately 3 percent of the average fruit drop in orchard A, shown in table 3, occurred during the 3 days just prior to the second picking on September 15.

Data in table 3 show that the average total fruit drop in orchard A was considerably less from the sprayed (7.3 percent) than from the unsprayed (27.9 percent) main leaders. Dropping of green fruit from either type of leader was not great. The difference in total drop, therefore, was largely the result of the ripened fruits being held on the hormone-sprayed halves. Fruit from orchard B was harvested at a less advanced stage of maturity than that of the second picking from orchard A and the drop of ripe fruits was also correspondingly less. Here, as in orchard B, the unsprayed fruits dropped more freely.

TABLE 3.—*Effect of α-naphthaleneacetic acid spray on the harvest drop and maturity of Bartlett pears, 1942*

Orchard and tree No.	Leader treatment	Original fruits	Fruit drop [1]			Total ripe fruits (dropped and picked) [1]
			Green	Ripe	Total	
Orchard A:		*Number*	*Percent*	*Percent*	*Percent*	*Percent*
No. 1	Sprayed	421	1.2	4.3	5.5	58.9
	Unsprayed	332	3.9	17.2	21.1	31.3
No. 2	Sprayed	429	2.8	.7	3.5	48.2
	Unsprayed	575	2.6	10.0	12.6	27.3
No. 3	Sprayed	524	1.9	9.9	11.8	80.2
	Unsprayed	585	6.3	40.5	46.8	54.6
Average [1]	Sprayed		2.0	5.3	7.3	63.7
	Unsprayed		4.3	23.6	27.9	38.9
Orchard B:						
No. 1	Sprayed	1,737	3.2	2.2	5.4	22.4
	Unsprayed	1,362	8.1	7.3	15.4	9.1

[1] Based on number of original fruits on the sprayed and unsprayed leaders.

Data in table 3 show that when Bartlett pears were harvested at an advanced stage of maturity the hormone spray stimulated the ripening of the fruit on the trees in both orchards. In orchard A, 63.7 percent of the original sprayed fruit (including drops and harvested fruit) ripened on the trees, whereas only 38.9 percent of the untreated fruit responded in a similar manner. Some of this difference in the amount of ripening on the trees under test may be accounted for in part by the fact that less ripe fruit dropped from the sprayed than from the control leaders.

After fruit requirements for the physiological storage studies were met, the remainder of the green fruit harvested from orchard A, on September 15, was ripened at room temperature (55° to 80° F.) for 6 days. Examination of the ripened fruit showed that core breakdown was present in 81.2 percent of the hormone-sprayed lot and in only 11 percent of the untreated lot. These figures are further evidence that the sprayed fruit was considerably more mature at harvest even though at that time both lots were green in color and firm.

Observations were also made on the dessert quality and condition of the Bartlett pears from orchards A and B after storage at 31° F. and after ripening at 65°. These data are shown in table 4. It will be seen that there was no appreciable difference in the storage response, dessert quality, or condition of the sprayed and the unsprayed lots of the first picking, made 8 days after the application of α-naphthaleneacetic acid. When harvested at an advanced stage of maturity (second picking orchard A) and ripened after short intervals of storage, both lots were inferior in dessert quality to the first picking. As judged by the amount of core break-down in the ripened fruit, the hormone-sprayed lots were more mature.

Fruit from orchard B was harvested at a stage of maturity intermediate to the two pickings in orchard A. When fruits were ripened for 5 days after storage at 31° F. for 3 weeks, the dessert quality and condition of both the sprayed and the unsprayed lots were satisfactory. They became mealy and overripe after 9 days at 65°, with core

break-down more severe in the hormone-sprayed fruit. When ripened after longer cold storage, dessert quality and condition became progressively inferior and core break-down also became more prevalent in the sprayed fruit.

TABLE 4.—*Dessert quality and condition of Bartlett pears, as influenced by the harvest application of α-naphthaleneacetic acid spray, after storage at 31° F. and ripening at 65°, 1942*

Orchard and picking	Storage period	Ripening period	Sprayed fruit	Unsprayed fruit
	Days	*Days*		
Orchard A (first picking, Aug. 25).	32	6	Juicy; prime flavor; no difference in ripening character attributable to spray.	Same as sprayed.
		9	Slightly overripe; no core break-down.	Do.
	51	5	Juicy; flavor good; no difference in ripening behavior attributable to spray.	Juicy; dessert quality good; ripening character same as sprayed.
		7	Dessert quality still mostly fair; some overripe; 14 percent showing traces of core break-down.	Dessert quality still fair; some slightly overripe; no core break-down.
	73	5	Somewhat granular; generally lacking in juiciness; dessert quality fair; no scald or core break-down.	Same general condition and dessert quality as sprayed fruit.
		7	Overripe; 45 percent core break-down.	Overripe; 33 percent core break-down.
Orchard A (second picking, Sept. 15).	11	4	Dessert quality poor; woody, tough texture; distinct lack of juice; 50 percent core break-down.	Same general condition and quality as sprayed lot; 21 percent core break-down.
	30	3	Colored full yellow and practically ripe upon removal from 31°; 66 percent core break-down at this time and 80 percent after ripening; quality poor.	Same general appearance as sprayed upon removal from 31°; but with only 26 percent core break-down; after ripening 56 percent core break-down; quality poor.
Orchard B (single picking, Sept. 3).	23	5	Juicy; prime flavor; dessert quality good; no apparent difference in ripening attributable to spray.	Very similar to sprayed in dessert quality and condition.
		9	Overripe; slightly mealy; no scald; 60 percent showing slight core break-down.	Overripe and mealy; slight core break-down in 48 percent of fruit.
	42	5	Texture somewhat tough, with loss of juiciness; ripened with fair quality; no scald or core break-down.	Dessert quality and condition very similar to sprayed.
		7	Overripe; no scald; 64 percent core break-down.	Overripe; no scald; 20 percent core break-down.
	64	5	Dessert quality fair to poor; slightly mealy; no scald or core break-down.	Same as sprayed.
		7	Mealy and overripe; 70 percent core break-down.	Mealy and overripe; 43 percent core break-down.

In conjunction with investigations on the effect of applying the hormone spray to Bartlett pears in orchards A and B, studies were also made of the physiological changes in various lots of these fruits during storage at 31° F. The results are presented in table 5. Data for the first picking from orchard A show that the application of the hormone spray did not influence the storage physiology of the fruit. In this respect the data complement and substantiate the observations on the ripening behavior of similar fruits shown in table 4.

TABLE 5.—*Physiological changes in Bartlett pears during storage at 31° F., as influenced by the harvest application of α-naphthaleneacetic acid, 1942*

Orchard, picking, and storage period (days)	Sprayed fruit				Unsprayed fruit			
	Firmness (pressure test)	Soluble pectin	Organic volatiles ($Ce(SO_4)_2$ reduced per 100 gm. of fresh tissue)	Respiration (CO_2 per kilogram-hour)	Firmness (pressure test)	Soluble pectin	Organic volatiles ($Ce(SO_4)_2$ reduced per 100 gm. of fresh tissue)	Respiration (CO_2 per kilogram-hour)
Orchard A (first picking, Aug. 25):	*Pounds*	*Percent*	*Milligrams*	*Milligrams*	*Pounds*	*Percent*	*Milligrams*	*Milligrams*
2	15.8	0.0387	59.8	3.72	16.2	0.0360	63.9	3.65
34	14.4	.0397	54.7	2.68	14.5	.0375	58.1	2.65
63				3.55				3.86
73	14.3	.0410	99.0	4.36	14.3	.0400	99.6	4.24
Average	14.8	.0398	71.1	3.58	15.0	.0378	73.9	3.60
Orchard A (second picking, Sept. 15):								
0	9.0	.2050	219.2		10.5	.1500	187.4	
13	8.1	.2450	228.7	3.89	10.5	.1500	162.0	3.58
29				4.23				3.88
36	8.1	.2750	272.4	4.41	8.3	.1950	277.3	3.95
Average	8.4	.2416	240.1	4.18	9.8	.1650	208.9	3.80
Orchard B (single picking, Sept. 3):								
6	12.1	.0512	78.0	2.72	13.0	.0460	76.4	2.56
15	11.5	.0715	66.4	2.62	13.0	.0430	56.0	2.39
54				3.60				3.35
68	11.4	.0890	132.8	3.77	12.3	.0525	116.2	3.47
Average	11.7	.0706	92.4	3.18	12.8	.0472	82.9	2.94

By the time of the second picking in orchard A and the single one in orchard B, the harvest application of the hormone spray had affected the storage physiology of the fruit. Data in table 5 show the sprayed fruit to be more mature than the unsprayed, because the average firmness was less and the soluble pectin, organic volatiles, and respiration rate were greater in the sprayed than in the unsprayed.

The data in tables 1, 2, and 5 show that α-naphthaleneacetic acid spray may affect the quantity of organic volatile materials produced in apples and pears. This effect is particularly evident in Delicious apples and Bartlett pears in which maturity at harvest was advanced most by the use of the hormone spray. At such times more volatiles were found in the hormone-sprayed fruit shortly after harvest. The failure of some of the fruit to show pronounced changes in volatile content may have been due to the fact that measurement of the volatile substances was not made at the proper stage of maturity to show the maximum influence of the hormone.

The soluble pectin content of the juice and the respiratory intensity of the fruit are among the most sensitive and informative of current indexes of maturity. They often reflect small differences in degree of ripeness between two lots of fruit that would escape detection by visual or organoleptic means. The present studies clearly indicate that, if harvest is delayed too long after the application of a hormone spray, sprayed Bartlett pears will mature faster on the tree and ripen faster during storage than comparable unsprayed fruit.

It has been suggested that the apparent stimulation of ripening of Bartlett pears by the hormone spray may have been an indirect effect, since it is possible that the removal of a portion of the crop (through drops) had a physiological effect on the rate of ripening of the fruit remaining on the tree. As a check on the results obtained from the use of hormone sprays during the previous year and an aid in the interpretation of these results, the work with Bartlett pears was continued during the season of 1943.

At that time a portion of the crop, comparable with the normal drop of the unsprayed fruit, was removed daily from the hormone-sprayed leaders. Differences in the rate of ripening and in the amount of core break-down from such a procedure were noted. The results of these experiments are shown in table 6.

TABLE 6.—*Effect of α-naphthaleneacetic acid spray on fruit drop, ripening, and core break-down in Bartlett pears, 1943*

Tree No. and leader treatment	Fruits dropped or removed [1]				Total ripe fruits (dropped and picked) [1]	Ripe fruits on tree at harvest [1]	Core break-down [2]
	Green drops	Ripe drops	Total drops	Removed to equal check drops			
No. 1:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Sprayed	4.9	2.5	7.4	22.1	34.5	39.7	40.1
Unsprayed	22.0	6.2	28.2		13.1	9.5	8.0
No. 2:							
Sprayed	5.4	3.7	9.1		40.6	40.5	62.8
Unsprayed	42.6	15.5	58.1		18.6	7.4	35.0
No. 3:							
Sprayed	2.7	4.2	6.9	23.4	35.7	43.5	44.1
Unsprayed	27.6	7.0	34.6		12.6	8.4	13.7
Average: [1]							
Sprayed	4.3	3.5	7.8		36.7	41.2	49.0
Unsprayed	30.7	8.9	39.6		14.2	8.4	15.3

[1] Based on the number of original fruits on the sprayed and unsprayed leaders (not shown in table).
[2] After storage at 31° F. for 2 weeks and ripening at 65° for 5 days; data are based on the number of sprayed and unsprayed fruits used in the storage experiments.

It will be seen that the hormone spray was again very effective in reducing the amount of fruit drop. Losses from the unsprayed leaders were 4 to 6 times as great as those from the hormone-sprayed portions of the tree. Confirming the results of the 1942 season, hormone sprays again increased the rate of ripening in pears permitted to hang on the trees until well past optimum maturity for best storage. Data in table 6 show that, when comparisons are based on the original number of fruits on the trees, there were from 2 to 3 times as many ripe fruit on the sprayed as on the unsprayed branches.

Removal of 22 to 23 percent of the crop from the hormone-sprayed leaders (at daily intervals to compensate for the normal drop from the unsprayed limbs) apparently did not alter the rate of ripening of the attached fruit. These results indicate that the hormone effects are localized in the individual fruit, stem, and spur tissues. Furthermore, the stimulation of the ripening processes by the hormone is a specific response to the chemical rather than a result of an altered physiology induced by the removal of a portion of the crop.

It will be recalled that the data in table 6 are based on Bartlett

pears harvested 151 days from full bloom. Maturity of the fruit at that time was too far advanced for commercial handling, notwithstanding the fact that over half of the fruit on the trees was firm and green. Harvest was purposely delayed in order to obtain maximum differences in ripening between hormone-sprayed and unsprayed fruit. The pears at that time showed a firmness of 12.8 pounds' pressure. Late commercial pickings would have been made possibly 1 week to 10 days earlier, and differences in ripening would have been proportionally less. In these studies the fruit that was green at harvest was held at 31° F. for a period of only 2 weeks prior to ripening at 65°. It was found that after the ripening period the amount of core break-down was significantly greater in the sprayed than in the unsprayed fruit. This fact, of course, is correlated with the advanced maturity of even the green-colored fruit from the hormone-sprayed leaders at harvest. In all probability, it is this latter condition which causes so much loss from core break-down when hormone-sprayed Bartlett pears intended for the cannery are permitted to hang on the trees too long in order to obtain an increase in tonnage.

BING CHERRIES

Sweet cherries are sold largely as fresh fruit in the markets of the Middle West and the Atlantic seaboard, where buyers place great reliance on the freshness and greenness of the stem in evaluating condition and determining price. Any treatment that would preserve these characteristics would be very useful to the industry. Hormone sprays are thought to delay or influence certain changes in the relation of the fruit stem to its spur tissue. Because of this fact experiments were made to determine whether greenness and freshness of the stems of sweet cherries during storage and subsequent handling could be correlated with harvest application of α-naphthaleneacetic acid.

The fruit on one-half of a normal sweet cherry tree of the Bing variety was sprayed with the growth chemical as described on page 192. Fruits from both the treated and the untreated halves of the tree were harvested 1 week after spraying. They were then stored for 10 days at 31° and 45° F. The amount of soluble solids and moisture in the stems and fruits of both lots at harvest and after storage are presented in table 7.

TABLE 7.—*Effect of a harvest application of α-naphthaleneacetic acid spray on stem moisture, soluble solids, and weight of Bing cherries stored for 10 days*

Storage temperature (°F.) and treatment	Stem moisture	Fruit	
		Soluble solids	Loss in weight
At harvest:	*Percent*	*Percent*	*Percent*
Sprayed	68.8	16.4	
Unsprayed	70.4	16.4	
31° storage:			
Sprayed	54.1		3.5
Unsprayed	54.0		3.4
45° storage:			
Sprayed	54.4		4.3
Unsprayed	53.7		4.3

These data show that the moisture relations in the stem and fruit of the Bing cherry were not markedly influenced by the application of the hormone spray. When the fruit was held at a relative humidity of 85 percent, the moisture content of the stems was not altered even by a difference of 14° F. in storage temperatures. Loss in weight of the fruit, however, was nearly 25 percent greater at 45° than at 31°. This loss apparently is correlated with the rate of respiration, since it has been found (*11*) that sweet cherries respire more than twice as fast at 45° as at 31°.

Examination of test lots of packed cherries after storage showed that both the hormone-sprayed and the unsprayed lots held at 45° F. were darker than those at 31°. There were no observable differences in the greenness or freshness of the stems between the sprayed and the unsprayed fruits at storage temperatures of either 31° or 45°. These observations are confirmed to some extent by the uniformity of the moisture percentages in the stems of the different lots (table 7).

Since neither the appearance nor the storage quality of Bing cherries was enhanced by the use of α-naphthaleneacetic acid, it is doubtful whether such sprays will be used commercially for sweet cherries, as fruit drop prior to harvest is not a problem in Bing, Lambert, or Napoleon (Royal Ann) varieties. Furthermore, it was found that when Bing cherries were sprayed with the hormone the adherence of the stem to the spur was greatly increased and picking without pulling the stem from the fruit became much more difficult.

DISCUSSION

Most of the published papers on the use of hormone sprays for controlling the harvest drop of apples have cautioned against picking the fruit at a too advanced stage of maturity. Batjer (*2*) recently summarized the current information on the proper use of these sprays and suggested that they should prove of greatest value to the fruit grower in affording protection against dropping before and during normal picking operations rather than through the extension of the harvesting period.

So far as the writers know, no experiments have been reported in which hormone sprays have increased the rate of ripening of apples when pickings were made from 1 to 2 weeks after application of the harvest sprays and the fruit had not become overmature prior to removal from the tree. In the present studies physiological measurements and storage observations showed that when Winesap and Delicious apples and Bartlett pears were harvested within their normal range of acceptable picking maturity the rate of ripening of the fruit was not increased by the use of the spray.

Part of the commercial crop of apples and pears is not always harvested at its proper stage of picking maturity. Much of the Bartlett pear crop is sold to canneries on a tonnage basis. Knowing that his fruit will increase in weight from 2 to 3 percent a day, the grower naturally wishes to delay picking in order to harvest a maximum tonnage. Under normal conditions, when Bartlett pears attain a firmness of 15 pounds' pressure, the rate at which they drop just about equals their rate of increase in weight. The grower then is forced to harvest his crop in order to avoid excessive losses. By the use of a

hormone spray he can reduce his fruit drop, and should he desire to postpone harvest he can pick his crop on the basis of maximum tonnage rather than at an acceptable maturity for subsequent storage. Unfortunately the increase in this latter practice has recently caused severe losses to pear canners.

The data presented in this study show that when Delicious apples and Bartlett pears treated with a hormone spray are permitted to hang on the trees much beyond the optimum harvest season, they will ripen faster than comparable lots of unsprayed samples. In other words, those physiological processes closely associated with ripening (pectin changes and respiratory activity) are directly stimulated by the specific action of the hormone spray. Fortunately, however, there is no distinguishable difference in the rate of this increase in maturity between hormone-sprayed and unsprayed fruit until they are well past the stage at which Delicious apples and Bartlett pears should normally be picked to meet optimum dessert and storage requirements.

Obviously some fruits on the tree mature faster than others. To a large extent these may constitute the normal drop. When a hormone spray is used, such fruits will be held on the tree and to that extent will contribute to the amount of total ripe fruit on the tree. This fact, however, is not a satisfactory explanation for the large difference in the amount of ripe fruit on sprayed as compared with unsprayed Bartlett pears as reported in these studies. Such a large difference in the amount of total ripe fruit at harvest, together with the increased amounts of soluble pectin, core break-down, and respiratory activity of the sprayed fruits, all suggest that the α-naphthaleneacetic acid was responsible in some manner for the increased ripening of the sprayed fruit.

Just when and how such a harvest spray as α-naphthaleneacetic acid accelerates the maturation processes in unpicked apples and pears is not known. Data presented in this study suggest that either a certain stage of maturity must be attained by the fruit or a certain period of time after application of the spray must elapse before the stimulatory action of the hormone becomes operative. That the effect of the spray is direct rather than indirect has been demonstrated in the Bartlett pear studies reported for 1943, in which it was found that the manual removal of a portion of the sprayed fruits comparable in number and maturity with the normal drop from the unsprayed leaders did not retard the accelerated rate of ripening of the hormone-sprayed fruits remaining on the trees.

Since these growth chemicals are effective in the control of the harvest drop of apples and pears, they must retard the normal rate of abscission changes within the stem and spur tissue of the fruit. Through quantitative chemical analyses Stuart (*15*, *p. 311*) was able to show a "directional shift of large amounts of nitrogen and carbohydrates" in the hypocotyls of kidney bean cuttings by treatment with indoleacetic acid. It is suggested that, in cases of accelerated ripening of apples and pears after the use of α-naphthaleneacetic acid, the mobilization of the food reserves may be influenced by the hormone either through its effect on the translocation channels in the stem and

spur tissues or through its inhibitory effect on the absciss region itself.

It has been reported (*1*) that α-naphthaleneacetic acid can be used successfully to retard the shriveling of the fruit and the browning of the stems of Montmorency cherries. The acid content of the fruit was decreased while the amount of sugar increased to the point where it was not necessary to add more in the making of cherry juice. These changes were said to have occurred on the tree over a period of 3 weeks after the application of the hormone spray at the close of the normal picking period. In the present study, when the application of the hormone spray was synchronized with commercial picking and handling operations, it was found that the growth chemical did not influence the turgidity, moisture content, or fresh appearance of the stems of Bing cherries. Total solids at harvest and loss in weight of the fruit during storage were not correlated with the presence of the hormone spray. In the light of these facts it is doubtful whether such sprays will find general commercial application in the sweet cherry producing districts in the Northwest.

SUMMARY AND CONCLUSIONS

In studying the influence of a hormone spray on the maturity and storage physiology of fruits, it was found that α-naphthaleneacetic acid greatly reduced the harvest drop of Winesap and Delicious apples and Bartlett pears.

When these fruits were harvested within their normal range of acceptable picking maturity from 1 to 2 weeks after application of the spray, apparently their storage physiology, quality, and degree of ripeness were not influenced by the hormone.

The results of these studies show that the fruit grower can safely use α-naphthaleneacetic acid spray for the prevention of fruit drop without impairing the keeping quality of Delicious apples and Bartlett pears, provided harvest is not postponed too long after the spray is applied and maturity is within the recommended picking range for the variety. However, if these conditions are ignored, this spray will increase the rate of ripening to the extent that losses from break-down following water core in apples and core break-down in pears may become serious. With Bartlett pears especially, the grower should harvest his sprayed fruit at the stage of maturity that will result in best storage quality rather than on the basis of maximum tonnage.

Maturity on the tree and ripening in storage were definitely increased by the hormone when Delicious apples and Bartlett pears were permitted to hang on the trees for an extended period after the application of the spray or until the fruit became slightly too mature for best storage. In these cases the soluble-pectin content, respiratory activity, and internal break-down of apples and core break-down in pears were much greater in the hormone-sprayed than in the unsprayed fruits. From two seasons' records it was found that when Bartlett pears were harvested at this stage of advanced maturity, ripened fruits (including dropped and picked) were approximately twice as numerous on the sprayed as on the untreated main leaders.

It is suggested that, when increased ripening does occur in apples and pears after the use of harvest sprays, such results are a direct

effect of the hormone. The mobilization of the food reserves may be influenced by the growth chemical either through its effect on the translocation channels in the stem and spur tissues or through its inhibitory action on the abscis region itself.

The moisture content, turgidity, and greenness of the stems of Bing cherries during storage were not correlated with harvest application of the hormone spray.

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